SELECTIVE MONOAMINE-OXIDASE INHIBITION IN MAN: A BIOCHEMICAL AND PHARMACOLOGICAL STUDY

Submitted for the degree of Doctor of Philosophy

by

John David Elsworth

Institute of Obstetrics and Gynaecology
University of London

1980

ABSTRACT OF THESIS

Much of the work described in this thesis centres on the selective MAO-B inhibitor (-)-deprenyl. Its inhibitory action was determined *in vitro* and a 10^{-6} M concentration was found to produce optimum selectivity between MAO-A and MAO-B. This concentration is approximately equal to 10 mg of the drug evenly distributed throughout an average body water space. When administered to normal volunteers, this dose of deprenyl produced rapid inhibition of platelet MAO activity. Urinary phenylethylamine, phenylacetic acid and dopamine were monitored during treatment; phenylethylamine output became elevated only when platelet activity was inhibited by more than about 80%. An increase in phenylethylamine excretion appears to provide a good index of overall body MAO-B inhibition.

In normal and parkinsonian subjects, deprenyl is free from the tyramine and phenylethylamine-potentiating effects on blood pressure characteristic of other irreversible MAO inhibitors. This lack of "cheese effect" persisted even during chronic deprenyl treatment.

Clinical studies confirmed that deprenyl is a useful, if limited, adjuvant to L-dopa therapy in Parkinson's disease. Although individual variation in susceptiblity to platelet enzyme inhibition was observed, such differences had no clinical counterpart; in any case, activity rapidly ceased with a dosage regimen of 10 mg daily. As in controls, deprenyl raised urinary phenylethylamine concentrations in parkinsonian patients, apart from those simultaneously receiving a decarboxylase inhibitor.

Despite wide variation in the distribution of MAO A and B activity in the body, dopamine was oxidised vigorously in all tissues. In the brain the striatum and accumbens contained the lowest A:B ratio and dopamine oxidation in these regions was most susceptible to deprenyl inhibition.

TMH was identified as a novel substrate for MAO-B in man.

Deprenyl was shown to be converted *in vivo* to methamphetamine and amphetamine. The antiparkinsonian properties of the drug are unlikely to stem from the pharmacological effects of these metabolites.

Contents

Page

1 Title page
2 Abstract of thesis
3 Contents
7 Tables and Figures
9 Acknowledgements
10 Abbreviations

11 Chapter One - General introduction
11 (i) Discovery of MAO
11 (ii) Substrate specificity
12 (iii) Other requirements for activity
12 (iv) Distribution
12 (v) Inhibitors of MAO
14 (vi) Function of MAO
16 (vii) Multiple forms of MAO
18 (viii) Selective inhibitors of MAO
20 (ix) The potential of selective inhibitors

22 Chapter Two - Methods
22 (i) Sample preparation
22 (a) Urine
22 (b) Plasma
22 (c) Platelets
23 (d) Tissue
23 (ii) MAO method
23 (a) Validity of the assay
25 (b) Assay without added inhibitor
26 (c) Assay with added inhibitor
26 (d) Separation of products from substrate
26 (iii) PEA method
27 (a) Extraction
27 (b) Derivatization
27 (c) Analysis
28 (iv) PAA method
28 (a) Plasma extraction
29 (b) Urine extraction
29 (c) Derivatization
30 (d) Analysis

Contents (cont.)

Page

30 (v) DA method

30 (a) Extraction

31 (b) Derivatization

31 (c) Analysis

32 (vi) Protein estimation

33 Chapter Three - Deprenyl and the "cheese effect"

33 (i) The "cheese effect"

35 (ii) Paradoxical absence of the "cheese effect"

37 (iii) Selective MAO inhibitors and the "cheese effect"

38 Methods

38 (i) Oral amine pressor tests

40 (ii) Urinary PEA, PAA and DA excretion before and after deprenyl

40 (iii) Effect of deprenyl on intestinal and platelet MAO activity *in vitro*

40 (iv) Effect of deprenyl on the metabolism of orally ingested amines

41 (v) Relationship between PEA excretion and MAO inhibition

41 Results

41 (i) TYR pressor test

45 (ii) PEA pressor test

45 (iii) Inhibition of MAO by deprenyl

45 (a) *In vivo*

45 (b) *In vitro*

51 (iv) Deprenyl and PEA excretion

51 (a) Effect on free amine

51 (b) Effect on conjugated amine

51 (v) Relationship between MAO inhibition and PEA excretion

51 (vi) TYR metabolism after MAO inhibition

56 (vii) PEA metabolism after MAO inhibition

56 (viii) Effect of deprenyl on PAA

56 (a) Plasma concentration

56 (b) Urinary concentration

56 (ix) Effect of deprenyl on DA excretion

56 Discussion

Contents (cont.)

Page

67 Chapter Four - Deprenyl in Parkinson's disease
67 (i) Aetiology
67 (ii) Pathology
68 (iii) Treatment
68 (a) L-dopa
69 (b) Other forms of treatment
70 (c) MAO inhibitors
72 Methods
72 (i) Clinical
73 (ii) Biochemical
73 Results
73 (i) Clinical
75 (ii) Biochemical
83 Discussion

90 Chapter Five - The effect of deprenyl on DA oxidation in human brain and peripheral tissues
90 (i) MAO in brain
91 (ii) Mixed substrate experiments
92 Materials
92 Methods
92 (i) Collection of material
93 (ii) Dissection of brains
93 (iii) Homogenization
93 (iv) MAO assay
95 Results
100 Discussion

105 Chapter Six - The metabolism of deprenyl
105 Introduction
107 Materials
107 Methods
107 (i) Determination of methamphetamine and amphetamine
108 (ii) Deprenyl assay
108 (iii) Plasma MAO inhibitor assay
110 (iv) Alteration of urinary pH
110 (v) Substitution of deprenyl for methamphetamine and amphetamine

Contents (cont.)

Page

111 Results

111 (i) GC-MS assay for methamphetamine and amphetamine

111 (ii) Quantification of deprenyl metabolism

111 (iii) The contribution of deprenyl metabolites to its therapeutic action

115 Discussion

121 Chapter Seven - TMH as a MAO-B substrate in man

121 (i) General introduction

121 (ii) Histamine as a central neurotransmitter

123 (iii) Histamine metabolism

126 (iv) The importance of TMH

128 (v) TMH as a substrate for MAO

129 Materials

130 Methods

130 (i) Direct assay of ^{3}H-TMH oxidation

130 (ii) The effect of deprenyl on ^{3}H-TMH oxidation

130 (iii) Substrate competition between PEA or 5HT and TMH

131 (iv) Counting and extraction efficiencies

131 Results

134 Discussion

140 Chapter Eight - General discussion

143 References

Publications

Tables and Figures

Page			
13	Figure	1:1	Structures of some MAO inhibitors
15	Figure	1:2	The relationship between some MAO substrates
19	Table	1:1	Specificity of some monoamine oxidase substrates
24	Figure	2:1	Effect of enzyme concentration on reaction velocity
42	Table	3:1	To show response of healthy normal volunteers to oral TYR
43	Figure	3:1	Effect of deprenyl on the pressor response to oral TYR
44	Table	3:2	To show response of patients with Parkinson's disease to oral TYR
46	Figure	3:2	Showing changes in platelet MAO activity and PEA excretion in volunteer 1 receiving deprenyl
47	Figure	3:3	Showing changes in platelet MAO activity and PEA excretion in volunteer 2 receiving deprenyl
48	Figure	3:4	Showing changes in platelet MAO activity and PEA excretion in volunteer 3 receiving deprenyl
49	Figure	3:5	Showing changes in platelet MAO activity and PEA excretion in volunteer 4 receiving deprenyl
50	Figure	3:6	_In vitro_ deprenyl inhibition of intestine and platelet MAO
52	Table	3:3	Effect of deprenyl on PEA excretion in normals
53	Table	3:4	Showing the variation in free PEA excretion in normals
54	Table	3:5	Comparing the effect of deprenyl on the excretion of free and conjugated PEA
55	Table	3:6	Relationship between platelet MAO activity and PEA excretion
57	Table	3:7	Effect of deprenyl on PEA and hydroxyphenylacetic acid excretion after PEA ingestion
58	Table	3:8	Effect of deprenyl on plasma PAA concentration
59	Table	3:9	Effect of deprenyl on urinary PAA concentration
60	Table	3:10	Effect of deprenyl on urinary DA excretion
74	Table	4:1	The response of "on-off" disabilities to deprenyl
76	Table	4:2	Frequency of side-effects
77	Table	4:3	Effect of deprenyl on platelet MAO activity and parkinsonism
79	Figure	4:1	Time course of inhibition of platelet MAO by oral deprenyl
81	Table	4:4	Free urinary excretion of PEA in Parkinson's disease
82	Table	4:5	Effect of carbidopa on free PEA excretion in Parkinson's disease

Tables and Figures (cont.)

Page		
84	Table 4:6	Effect of deprenyl on free PAA excretion in Parkinsonians
94	Figure 5:1	Location of coronal section of brain which reveals the nucleus accumbens
96	Figure 5:2	Comparison of deprenyl inhibition of PEA and 5HT oxidation by human caudate
97	Table 5:1	5HT, PEA and DA-oxidising activities in different regions of human brain and inhibition of DA oxidation by 10^{-6} M deprenyl
98	Table 5:2	5HT, PEA and DA-oxidising activities in various peripheral tissues and inhibition of DA oxidation by 10^{-6} M deprenyl
99	Figure 5:3	Lineweaver-Burk plots to compare the Km values of placenta and platelet with DA as substrate
106	Figure 6:1	Structures of deprenyl, methamphetamine and amphetamine
109	Table 6:1	Comparitive electron-capturing ability of pentafluorobenzamides
112	Figure 6:2	Showing typical traces obtained in the GC-MS assay of methamphetamine and amphetamine
113	Table 6:2	Urinary output of methamphetamine and amphetamine in normal subjects
114	Table 6:3	The presence of a MAO inhibitory substance in plasma after deprenyl administration
116	Table 6:4	The effect of urinary pH on methamphetamine and amphetamine excretion in parkinsonian patients
124	Figure 7:1	Pathways of histamine metabolism
132	Figure 7:2	TLC analysis of ^{3}H-TMH metabolism to ^{3}H-MIAA by human liver
133	Figure 7:3	The sensitivity of PEA and TMH oxidation to deprenyl inhibition
135	Figure 7:4	Competitive inhibition of PEA oxidation by TMH
136	Figure 7:5	Effect of TMH on 5HT oxidation

Acknowledgements

I should like to thank Professor Sandler particularly, for all of his advice during my stay at the laboratory.

I am also grateful to all the other members of the department for their friendship and encouragement, and in particular to Dr. Graham King and Mr. Brian Pettit for assistance with the mass-spectrometric analyses.

I am indebted to Dr. Gerald Stern and his team at University College Hospital, especially to Drs. Andrew Lees, Christopher Ward and Cecily Lander, for conducting the clinical aspects of the work.

Finally, I wish to thank all my family, especially my mother, for constant support throughout my education.

Abbreviations

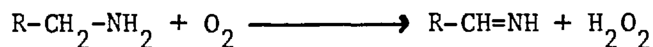
A	adrenaline
AAAD	aromatic amino acid decarboxylase
ADH	antidiuretic hormone
BZ	benzylamine
COMT	catechol-O-methyltransferase
CNS	central nervous system
d	days
D_7PAA	heptadeuterated phenylacetic acid
DA	dopamine
deprenyl	(-)-deprenyl, laevodeprenyl
GC	gas chromatograph(y)
GC-MS	gas chromatography-mass spectrometry
h	hours
HA	histamine
HD	histidine decarboxylase
HIS	histidine
HMT	histamine methyltransferase
5HT	5-hydroxytryptamine
IAA	imidazole acetic acid
MAO	monoamine oxidase
MIAA	methylimidazole acetic acid
min	minutes
NA	noradrenaline
PAA	phenylacetic acid
PEA	2-phenylethylamine, β-phenylethylamine
PFB	pentafluorobenzyl chloride
PFP	pentafluoropropionic anhydride
SAH	S-adenosylhomocysteine
SAM	S-adenosylmethionine
sec	seconds
TEA	tolylethylamine, para-methylphenylethylamine
TLC	thin layer chromatography
TMH	_tele_-methylhistamine
w	weeks
$^w/_v$	weight in volume

(i) Discovery of MAO

In 1928, Hare published her findings on an enzyme in liver capable of oxidising TYR, which she named "tyramine oxidase". In the next decade Blaschko et al (1937) and Pugh and Quastel (1937) identified enzymes able to oxidise A and aliphatic monoamines, which they called "adrenaline oxidase" and "aliphatic amine oxidase" respectively. It was soon realized, however, that these discoveries had each shed light on different aspects of one enzyme, given the name monoamine oxidase by Zeller (1951) and later classified as monoamine oxidase (monoamine: oxygen oxidoreductase (deaminating) EC 1.4.3.4)

(ii) Substrate specificity

It carries out the following reaction:



$$R\text{-}CH_2\text{-}NH_2 + O_2 \longrightarrow R\text{-}CH{=}NH + H_2O_2$$

$$R\text{-}CH{=}NH + H_2O \longrightarrow R\text{-}CHO + NH_3$$

The hydrogen peroxide is usually broken down in the presence of catalase in the following manner:

$$H_2O_2 \longrightarrow H_2O + \tfrac{1}{2}O_2$$

so that the overall reaction is:

$$R\text{-}CH_2\text{-}NH_2 + \tfrac{1}{2}O_2 \longrightarrow R\text{-}CHO + NH_3$$

The substrate specificity of MAO has been thoroughly investigated (Blaschko, 1952). It will oxidise primary, secondary and tertiary monoamines of the formula $R^1\text{-}CH_2\text{-}NR^2\text{-}R^3$ where R^2 and R^3 may be hydrogen atoms or methyl groups. In addition, the amino group must be attached to an unsubstituted methylene group. Of the aliphatic amines methylamine is not oxidised, and there is an optimum chain length of between five and six carbon atoms. MAO will not accept as substrates short chain diamines unless a distance of seven carbon atoms separate the amino groups.

This wide specificty overlaps with that of some other

amine oxidases, an aspect which has been reviewed by Blaschko (1966). These other enzymes are diamine oxidase, the plasma amine oxidases and the connective tissue monoamine oxidases. None has the ability to oxidise the MAO substrates DA, PEA, or 5HT in human tissue, compounds which are dealt with in detail in this thesis.

(iii) Other requirements for activity

Oxygen is the second substrate for MAO, but Tipton (1972) determined experimentally that at amine concentrations of 10 µm or less, the activity of the enzyme is relatively insensitive to quite large fluctuation in local concentrations of oxygen.

The oxidation of monoamines *in vivo* is depressed in rats deprived of certain nutrients. Extrapolating from such data (Sourkes and Missala, 1976) it seems that riboflavin and iron, but not pyridoxine and copper are required by MAO for full activity. In fact, covalently-bound flavin adenine dinucleotide is known to be located at the active site of MAO. Furthermore, Maycock et al (1976) found that the acetylenic inhibitors of the enzyme bind with the flavin moiety.

(iv) Distribution

MAO is widely distributed throughout the animal body, with particularly high concentrations occurring in the liver, kidney and intestines (Davison, 1958). Within the cell, it is an insoluble component of the outer membrane of the mitochondrion (Schnaitman and Greenawalt, 1968). In brain the enzyme has been found to be present in synaptosomes (Rodriguez de Lores Arnaiz and De Robertis, 1962), besides being located extraneuronally in glial cells (Silberstein et al, 1972). In a number of innervated tissues a substantial proportion of the enzyme has been identified extraneuronally (Klingman, 1966).

(v) Inhibitors of MAO

The great interest which now exists in the field of MAO inhibition began in 1951 when it was noticed that certain patients with tuberculosis became euphoric as a result of treatment with iproniazid (Figure 1:1). As this drug was found by Zeller and Barsky (1952) to be an inhibitor of MAO and

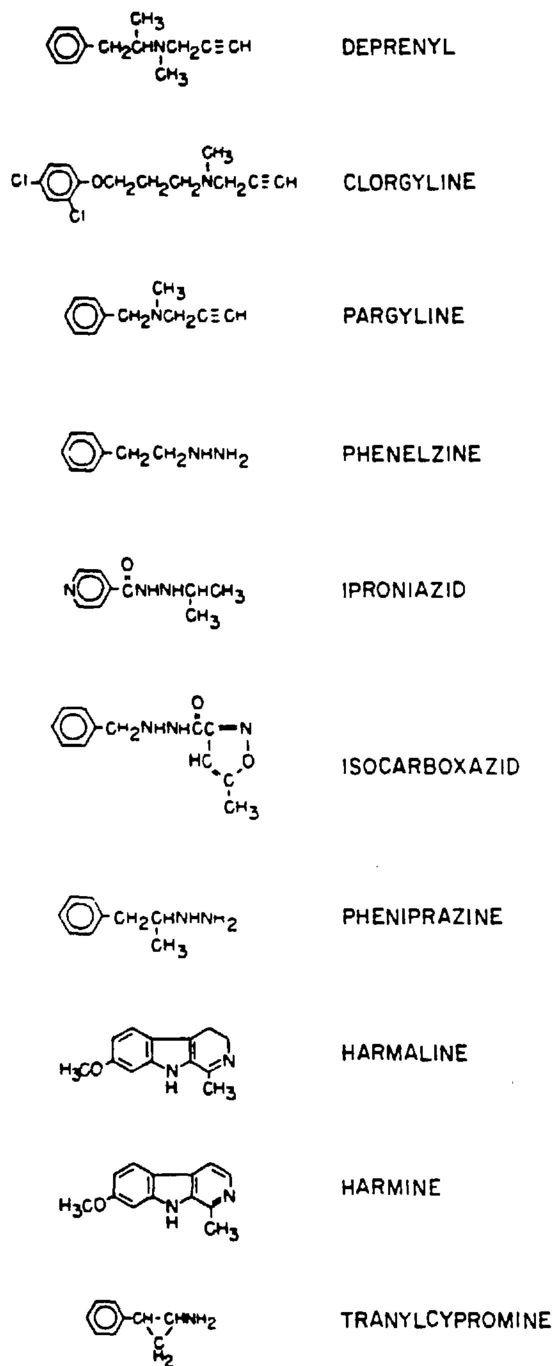


Fig. 1:1 Structures of some MAO inhibitors.

as the enzyme was known to be responsible for the oxidation of NA and 5HT (Figure 1:2), the basis of the amine hypothesis of mental depression was laid (Schildkraut, 1965). Indeed, iproniazid was used successfully in depression (Loomer et al, 1957), and other inhibitors were synthesized soon afterwards (Figure 1:1) as interest in MAO inhibitors rapidly developed. Such drugs were used in attempts to relieve many conditions which could conceivably be attributed to a monoamine insufficiency. In fact, many successes were noted, for example, in hypertension (Stern, 1963), migraine (Anthony and Lance, 1969) and Parkinson's disease (see Chapter 4).

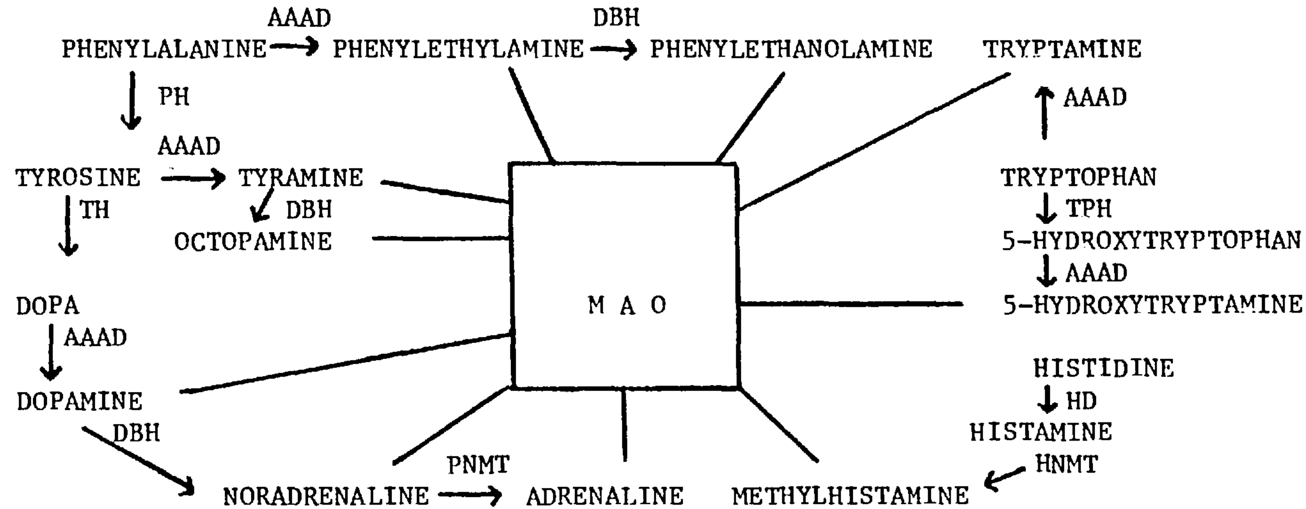
However, the MAO inhibitors fell into disrepute in the 1960s when their therapeutic effect in depression could not be demonstrated (Medical Research Council, 1965)—in a trial later acknowledged to be inadequate (e.g. Pare, 1976; Robinson et al, 1973)—and when serious side effects came to light (Blackwell, 1963a). Confidence in these drugs was undermined particularly by reports of fatalities due to the concomitant ingestion of certain foodstuffs or drugs. The resulting interactions resulted in acute hypertensive responses causing anything from mild headache in some patients to death from cerebral haemorrhage in others. The substances which provoked these attacks were analysed, and their amine or amino acid content was pinpointed as the cause of the toxic interaction (Blackwell and Marley, 1966). The most important was TYR present in high concentrations in cheese, and the side effect produced by the combination of amine-rich foods and MAO inhibitors, therefore, became known as the "cheese effect" (Chapter 3).

(vi) Function of MAO

The particularly high activity of MAO in organs such as liver, stomach and intestine (Davison, 1958) suggests a protective role for the enzyme against exogenous monoamine substrates. At these sites it would destroy potentially dangerous amines which have been ingested. Thus the "cheese effect" is thought to stem from the entry into the general circulation of large amounts of TYR which bypass

Figure 1:2

The relationship between some MAO substrates



Enzyme abbreviations:

MAO	Monoamine oxidase
AAAD	L-aromatic amino acid decarboxylase
DBH	Dopamine-β-hydroxylase
PH	Phenylalanine hydroxylase
TH	Tyrosine hydroxylase
TPH	Tryptophan hydroxylase
PNMT	Phenylethanolamine-N-methyl transferase
HD	Histidine decarboxylase
HNMT	Histamine-N-methyltransferase

the normal enzyme barrier and proceed to release NA from nerve endings on to adrenergic receptors of blood vessels. The subsequent constriction of the vasculature manifests as a rise in blood pressure.

In neural tissues, the role of MAO within the presynaptic nerve is probably to catabolise released monoamine transmitters after their reuptake into the neurone, and to inactivate transmitter which leaks from its intraneuronal storage site. That such an important role in transmitter regulation rests on MAO becomes apparent after MAO is inhibited, when an increase in the concentration of catecholamines and 5HT in neurones occurs (Tozer et al, 1966).

Even though reuptake ("$Uptake_1$") of catecholamines is the primary mechanism for terminating their action in the synapse, Trendelenburg et al (1972) demonstrated that this action is governed indirectly by MAO. Under conditions of MAO inhibition, free intraneuronal amine concentration rises to such an extent that the uptake system can no longer function against the adverse concentration gradient.

The purpose of MAO which is not intraneuronal is less clear. However, an extraneuronal uptake system ("$Uptake_2$") has been identified (see Iversen, 1973) and MAO presumably oxidises amines taken up in this way. Although $Uptake_2$ probably plays a minor role in the inactivation of NA normally, under conditions of $Uptake_1$ blockade, $Uptake_2$ may assume more importance (Langer, 1970).

Other types of function have been assigned to MAO. For example, the thyroid gland enzyme has been postulated as a hydrogen peroxide generating system for iodothyronine systhesis (Fischer et al, 1966). It has also been suggested that the deaminated metabolites produced in the MAO reaction are not without physiological importance. A role has been proposed for them as regulators of cellular oxidation reactions (Gorkin and Orekhovitch, 1967) and as controllers of sleep mechanisms (Jouvet, 1969).

(vii) Multiple forms of MAO

Even though the clinical use of MAO inhibitors had fallen

out of favour, the early successes which were achieved ensured that research in this field was not deterred. Indeed, much interest was generated when Youdim and Sandler (1967) and Kim and D'Iorio (1968) used electrophoretic techniques to separate solubilized preparations of MAO into a number of discrete bands of enzyme activity. Each possessed different activities towards substrates and different susceptibilities towards inhibitors (Youdim, 1972). It was thought at the time that these constituents might reflect the presence of distinct isoenzymes which also existed *in vivo*. This was not the first time that experimental evidence had indicated multiple forms of MAO. As long ago as 1943, Alles and Heegard reported that various substrates were oxidised at different rates by liver MAO, and in 1955 Satake actually suggested that MAO comprised an interrelated group of enzymes, each with different substrate preferences. In 1968, Johnston, instead of attempting physically to separate MAO, classified the enzyme into two forms based on their relative sensitivities to the inhibitor clorgyline (Figure 1:1). He identified a clorgyline-sensitive species which preferentially deaminates 5HT and TYR, and a clorgyline-resistant species which actively deaminates BZ and TYR. These two variants he called type A and type B respectively. This division of the enzyme into two forms has gained widespread acceptance as the working model of MAO. Its credibility was much enhanced when it was found that the inhibitor, deprenyl (Figure 1:1), affects the type B MAO selectively (Knoll, 1976). Considerable evidence has accummulated, pointing to the actual *in vivo* existence of these two forms, unlike the electrophoretically separated forms which, it became apparent, do not reflect the situation in the cell. Rather they appear to arise as a consequence of the procedures needed to solubilize the membrane - bound enzyme. These techniques were shown to produce species with various amounts of membrane material still attached (Houslay and Tipton, 1973), which therefore migrated at different rates. Houslay and Tipton

(1973) also demonstrated that treatment of the solubilized preparation with chaotropic agents, which would separate the lipid material from the enzyme, abolished the electrophoretic multiplicity. Removal of the lipid in this manner also resulted in abolition of substrate preferences, yet the enzyme activity was resistant to clorgyline and sensitive to deprenyl. Thus even though such a preparation cannot be used as a model of the enzyme, it does indicate that the selectivity of the inhibitors of MAO depends at least in part on the lipid environment of the enzyme (Houslay, 1977).

(viii) Selective inhibitors of MAO

Johnston's experiences with clorgyline (1968) were not the first time inhibitors had been observed to exert a discriminative effect on the oxidation of substrates. Hardegg and Heilbronn (1961) found that iproniazid inhibited 5HT and TYR deamination to different extents, and they proposed that these two amines were not substrates for the same MAO in rat liver. In 1962, Long demonstrated that 5HT oxidation was inhibited at least a hundred times more effectively than tryptamine, TYR and PEA by harmaline (Figure 1:1). Zeller (1961, 1963) noted the selective inhibitory effects of tranylcypromine (Figure 1:1) and pargyline (Figure 1:1) and such reports became increasingly more common in the following few years (see Fuller, 1972). Johnston's (1968) contribution was to bring some degree of order to a confused literature. In the wake of his study came many reports which confirmed and extended the concept. The two forms have been found in various proportions in different tissues ranging from the pure MAO-A in placenta (Egashira, 1976) to the platelet which contains exclusively MAO-B (Donnelly and Murphy, 1977). The list of substrate preferences has also been expanded: besides 5HT the A-form also oxidises NA and A (Houslay and Tipton, 1974) whereas the B-form, apart from BZ, oxidises PEA (Yang and Neff, 1973), phenylethanolamine (Edwards, 1978) and TMH (see Chapter 7). A number of substrates can be oxidised by both forms (see Table 1:1).

Table 1:1

Specificity of some monoamine oxidase substrates

Oxidised by A form	Oxidised by both forms	Oxidised by B form
5-hydroxytryptamine	dopamine	phenylethylamine
adrenaline	m-tyramine	*tele*-methylhistamine
noradrenaline	p-tyramine	benzylamine
3-methoxytyramine	octopamine	o-tyramine

However, latterly, irregularities in the simple binary classification have been scrutinised and collated (Fowler et al, 1978; Murphy, 1978). Some of the most notable deviations were reported by Squires (1972). He found, for example, that whereas clorgyline and harmaline, MAO-A inhibitiors, acted in the expected manner on enzyme from several rabbit tissues, the MAO-B inhibitors deprenyl and pargyline, exhibited no selectivity. His inhibitor studies also indicated the absence of MAO-A in all pig organs, except the intestine, yet the pig can still oxidise MAO-A substrates. Williams et al (1975) found it necessary to propose the existence of a further form, MAO-C, in order to explain the MAO activity of the rat circumventricular region. It now seems likely though that this derives from a particularly high concentration of MAO-B (Kim et al, 1979). Sources of some other anomalies have recently been uncovered. PEA, long regarded as being entirely specific for MAO-B, is in fact a substrate also for MAO-A, at the high concentrations used by many workers (Lewinsohn et al, 1980). BZ is a more specific substrate for MAO-B, but it is also oxidised by benzylamine oxidase ("plasma amine oxidase") so that deprenyl-resistant benzylamine oxidising activity should not be ascribed automatically to MAO-A.

It is now clear that a simple division of MAO into A and B forms can be misleading. Even though thermostability studies have been critisised (Achee et al, 1977), it may be that Squires (1972), interpreting data from such experiments, was correct when he concluded that both forms of MAO are heterogeneous. More recently, Fowler et al (1978) suggested that the A and B forms may represent families of enzyme species whose different properties arise from their lipid environment.

(ix) The potential of selective inhibitors

Despite the lack of clarity concerning the nature of MAO isoenzymes, it is apparent that at least two forms exist and that clorgyline and deprenyl inhibit the oxidation of only certain amines.

One of the driving forces behind the research into the inhibitors and multiplicity of MAO, was the hope that the clinical use of selective inhibitors would be safe from the "cheese effect" by allowing the oxidation of TYR to proceed, whilst being effective by inhibiting the oxidation of amines involved in various diseases.

If a MAO inhibitor could be found whose safety was beyond doubt, this class of drugs might reemerge to take a place in the clinicians therapeutic armoury.

From behind the Iron Curtain came claims that deprenyl was such a drug. It became possible to test this in 1976 as a result of a gift of deprenyl from Professor J. Knoll.

The general aims of the work reported in this thesis were, therefore, primarily to investigate the safety and usefulness of deprenyl.

CHAPTER TWO - METHODS

Methods employed repeatedly are described in this section, whilst those used more specifically are included in the appropriate chapter.

Unless otherwise specified, all chemicals used were of the highest purity available, usually purchased from either BDH Ltd., Poole, U.K. or Sigma Chemicals Company, Kingston-on-Thames, U.K.

(i) Sample preparation

(a) Urine

Samples were collected over 6N HCl (1 ml per h of collection). After the specimen was complete, the whole measured volume, or a 50 ml aliquot, was frozen at -20°C.

(b) Plasma

Whole blood was collected into tubes containing lithium heparin. The plasma was separated by centrifugation at 1500 g for 10 min, and stored at -20°C until assay.

(c) Platelets

The method of Challacombe et al (1971), slightly modified, was used. Venous blood (10 ml) was collected into 10 ml of 2% EDTA (w/v) in normal saline, and gently mixed. Isolation of platelets proceeded as soon as possible, but when a delay of several hours was necessary, samples were kept at 4°C. Storage in this manner for as long as 18 hours does not result in loss of MAO activity (Glover, unpublished data). The next step involved centrifugation at 250 g for 6 min, following which the platelet-rich plasma was separated and spun at 1500 g for 15 min. The supernatant was discarded and the platelet deposit teased away from contaminating red cells and resuspended in 1 ml 0.3M ice-cold sucrose. This suspension was spun at 1500 g for 15 min, the supernatant again discarded and the platelets taken up into sucrose as before. This fraction was vortex mixed for 5 sec to homogenize the platelets and then stored at -20°C.

Duplicate 0.02 ml portions (occasionally 0.05 ml if a poor yield of platelets was obtained) were used for protein estimation and MAO assay.

(d) Tissue

Depending on the tissue, homogenates from 10 to 50 mg were prepared in 1 ml 0.01M phosphate buffer, pH 7.4, using an Ultra Turrax homogenizer. By boring a hole the size of the Ultra Turrax shaft in the cap of the tube in which tissue and buffer were contained, a sealed system for homogenization was achieved for safety purposes. Homogenization was performed in a fume cupboard, as a further precaution against the risk of air borne particles of human tissue origin.

The homogenate was initially frozen in solid carbon dioxide and then stored at -20°C until assay.

(ii) MAO method

The assay used is a modification of that described by Robinson et al (1968). This procedure employs an ion-exchange column to separate substrate from product; although the present work was conducted initially in like manner, it was superseded by an organic solvent extraction procedure.

(a) Validity of the assay

The following steps were taken in order to validate and standardize the procedure.

Thorough homogenization of all cellular material and the subsequent freezing and thawing to which it was subjected, ensured that permeability barriers did not exist.

The linearity of the assay with respect to time of incubation and enzyme concentration has previously been established (Robinson et al 1968; White and Wu, 1975; Tipton and Youdim, 1976; Glover et al, 1977). However, to confirm that this was also true in the present series of investigations, the effect of enzyme concentration on reaction velocity was examined (Figure 2:1). A linear relationship was found over the range used in the subsequent work. This result also indicated that the reaction time-course is linear.

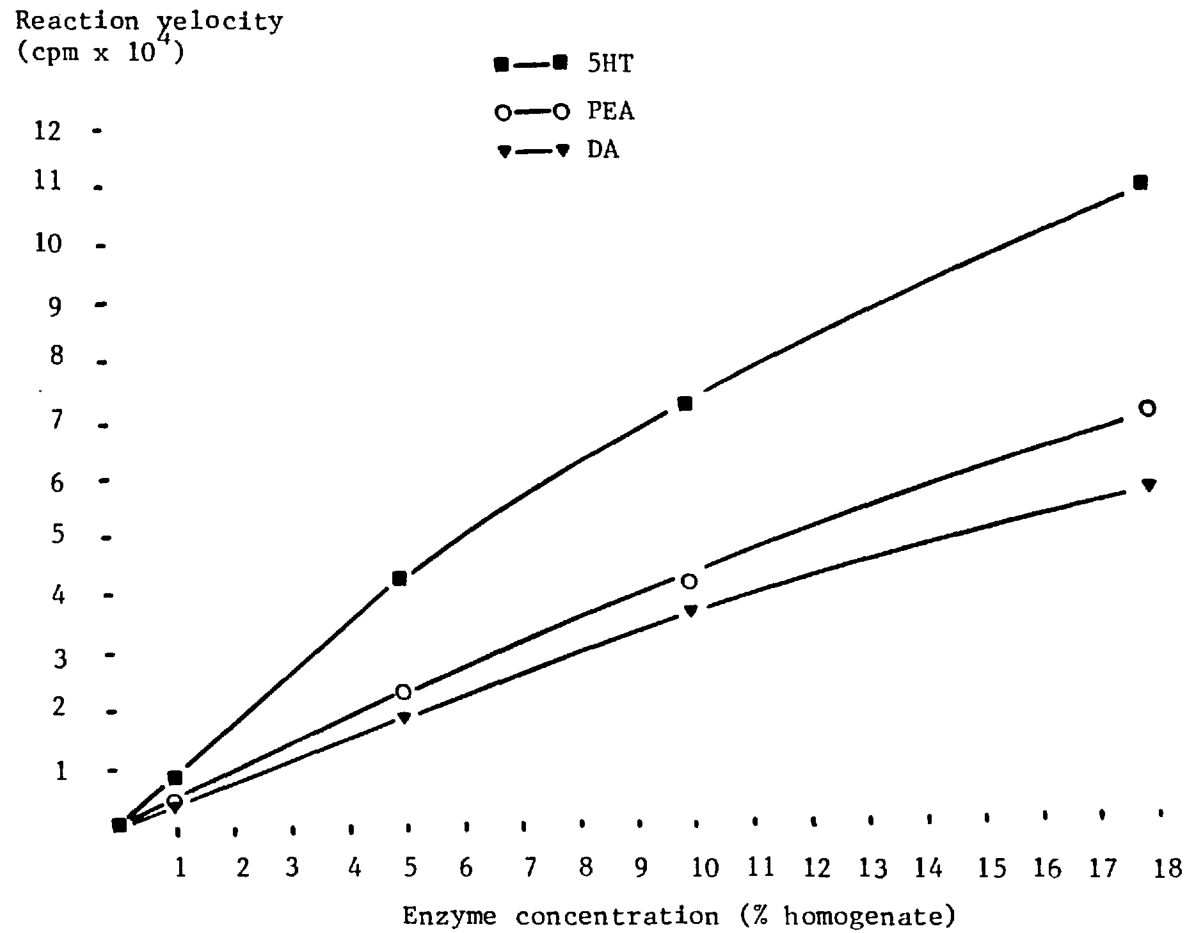


Fig. 2:1 Effect of enzyme concentration on reaction velocity

Human liver MAO activity with PEA (500 μM), DA and 5HT (600 μM).

The solvent extraction efficiencies for the separation of amines and products were determined by repeated extraction with the different substrates; 5HT (90%), DA (82%) and PEA (95%). An ion-exchange resin was formerly used for the separation of PEA from its deaminated products, and although its efficiency varied slightly between batches, it was generally also about 95%. Appropriate corrections to the estimated MAO activity values were made.

Although the radioactive substrates are tested for purity by the manufacturers, each batch was checked for extractable labelled contaminants which would upset the assay.

Radioactive substrates were diluted with cold substrate so that the final concentration in the reaction mixture was well above its particular Km value. For the assay of platelet MAO activity, the PEA concentration was 25 μM; for tissue analysis, substrate concentrations were: PEA 125 μM, 5HT and DA 300 μM.

Oxygen is the second substrate for MAO; its concentration was maintained by conducting the assay in open tubes held in a shaking waterbath.

(b) Assay without added inhibitor

Materials

^{14}C-PEA-New England Nuclear Corp., Boston, U.S.A.

Instagel-Packard Instruments Co., Caversham, U.K.

^{14}C-5HT and ^{14}C-DA-Radiochemical Centre, Amersham, U.K.

Method

0.02 ml of the enzyme preparation was added to 0.1 ml 0.1M phosphate buffer, pH 7.4, in plastic tubes on ice. 0.02 ml of either 10^{-2} M deprenyl solution, for blanks, or water for samples, was then added. After addition of 0.02 ml of radioactive substrate, the rack of tubes was transferred to a shaking waterbath at 37°C for 30 min. The reaction was stopped by plunging the tubes into an ice bath and adding 0.1 ml 2 M citric acid, if solvent extraction was to be used, or 1 ml ice-cold 0.1 M phosphate buffer, pH 7.4, for column extraction.

(c) Assay with added inhibitor

The procedure in (b) above was used except for the following: 0.02 ml of the inhibitor solution was added to the samples instead of 0.02 ml water and the tubes preincubated at room temperature for 30 min.

(d) Separation of products from substrate

In the ion-exchange technique, Amberlite CG50 (H) type I (100-200 mesh) was the resin employed. It was prepared for use by the method of Pisano (1960). Briefly, this involved converting the resin to the sodium form, then to the hydrogen form and finally back to the sodium form. The pH of the resin was adjusted to between 6.2 and 6.3 and it was then stored at 4°C until use.

Columns of resin (3 cm), were prepared in pasteur pipettes (0.5 cm diameter) plugged with glass beads. Each was washed with 2 ml water before the reaction mixture was applied and allowed to drain into a scintillation vial. The column was then washed with 2 ml water, which was collected into the same vial after passing through the resin. Instagel (scintillator solution) (10 ml) was added to the vial and, after mixing well, the contents were counted (Packard Tri-carb Liquid Scintillation Counter 3330).

In the solvent extraction technique, deaminated products of 5HT and DA were separated into 3 ml ethyl acetate-toluene (1:1) but for PEA, 3 ml toluene alone was used.

These solvents were added once the reaction had been terminated. The tubes were then capped and extraction achieved by shaking for 5 min. After centrifuging for 2 min at 1500 g, the aqueous layer was selectively frozen (-20°C), enabling the upper organic phase to be poured off into a scintillation vial. Instagel (3 ml) was added and the radioactivity then counted.

(iii) PEA method

Materials

TEA - Aldrich, Gillingham, U.K.; pentaflurobenzoyl

chloride - Pierce and Warriner, Chester, U.K.;
OV-225 - Applied Science Labs., State College, U.S.A.
PPE-21 - Supelco, Bellefonte, U.S.A.

The procedure used was essentially that of Blau et al (1979), which is very specific, unlike many of the early methods now considered to produce erroneously high values (Schweitzer et al, 1975; Reynolds, 1979).

(a) Extraction

Urine (2 ml) was adjusted to a pH in excess of 12 with 2 M NaOH, checked with indicator paper. After the solution had been saturated with NaCl, 2 ml of n-hexane was added and the sample vortex mixed for 30 sec. The phases were separated by centrifugation at 1500 g for 5 min. The upper organic phase was removed and the amines it contained back-extracted into 0.2 ml 1 M HCl by vortex mixing for 30 sec. Following centrifugation at 500 g for 1 min, the lower aqueous layer was transferred to a 1 ml screw-cap glass vial and freeze-dried.

If the conjugated form of the amine was to be measured, 6 M HCl was first added to the urine to reduce the pH to 1 and the sample, in a stoppered tube, was immersed in a boiling water bath for 1 h. After cooling, enough 6 M NaOH was added to raise the pH to 12. The procedure then continued as described above.

(b) Derivatization

To the residue was added 0.05 ml freshly prepared 2% pentafluorobenzoyl chloride in dried diethyl ether. The reaction proceeded for 2 min before excess reagent was removed under vacuum. n-Heptane (0.05 ml) and 0.02 ml ammonia were added, the vials capped, and the contents thoroughly vortex mixed.

(c) Analysis

Heptane layer (2 µl) was injected into a Hewlett-Packard 5173A gas chromatograph equipped with a ^{63}Ni electron-capture detector. Two types of column were used

during the course of the investigation; firstly 6 ft 3% OV-225 and later, because of better peak shapes, 6 ft 3% PPE-21. Both were run isothermally at 230°C with the carrier gas, 5% methane in argon, flowing at 30 ml min^{-1}. The injection port temperature was maintained at 300°C and the detector at 350°C.

Quantification was achieved by adding TEA to the urine prior to extraction to serve as an internal standard. As it was carried through the whole procedure it was subjected to the same manipulations as endogenous PEA and behaved like PEA during extraction and derivatization, but was separated from it by gas chromatography.

Every batch analyzed contained a reagent blank in which 2 ml H_2O was carried through the entire procedure.

The ratio of the peak heights (PEA/TEA) was calculated in each sample, and the absolute amount of PEA present was determined from a calibration curve which was constructed freshly each time a batch of samples was analysed. The worst correlation coefficient for this standard curve was 0.996 (5 data points); usually, it was 0.999.

(iv) PAA method

Materials

D_7 PAA - Merck, Sharp and Dohme Ltd., Hoddesdon, U.K.;
PFB - Digby Chemical Service, London, U.K.;
OV-1 - Applied Science Labs, State College, U.S.A.

The method of Fellows et al (1978) was followed for the determination of plasma concentration and this was adapted for measurement of the urinary compound.

(a) Plasma extraction

D_7 PAA (50 ng) in 0.1 ml H_2O was added to 0.5 ml plasma as an internal standard. This was diluted with 2.5 ml H_2O and protein precipitated by adding 2.0 ml 1 M perchloric acid. After mixing well, the precipitate was centrifuged down at 1500 g for 10 min. The supernatant was removed and 2 ml 1 M phosphate buffer, pH 6.5, added to it. The pH of the

mixture was adjusted with 2 M KOH to 6.0. Enough NaCl was then added to saturate the solution and the PAA extracted into an organic phase by adding 6.5 ml ethyl acetate and vortex mixing for 30 sec. The phases were separated by centrifugation at 1500 g for 10 min, and 5 ml of the organic layer was transferred and evapourated to dryness in a stream of nitrogen at 50°C in the presence of 0.05 ml triethylamine. The incorporation of triethylamine is important because it stabilizes the volatile acid by forming a salt. The residue remaining was transferred to a 1 ml screw-cap glass vial with 0.2 ml ethanol plus a drop of triethylamine. The contents of the vial were evapourated to dryness in vacuo.

(b) Urine extraction

D_7 PAA (1 μg) in 0.1 ml H_2O was added to 1 ml urine. This was acidified with 0.05 ml 6 N HCl and saturated with NaCl before adding 10 ml diethyl ether to extract the acid. After vortex mixing for 30 sec and centrifuging at 1500 g for 5 min, 0.5 ml of organic phase was transferred to a 1 ml screw-cap glass vial and 0.05 ml triethylamine added. The contents of the vial were dried in a stream of nitrogen.

When the concentration of the conjugated form was to be measured, 10 μg D_7 PAA in 0.1 ml H_2O was added to 0.1 ml urine. This was diluted with 0.5 ml H_2O and acidified with 0.5 ml concentrated HCl. The conjugate was then hydrolysed by heating the mixture in a boiling water bath for 2 h. The cooled hydrolysate was diluted with 3 ml H_2O and partially neutralized with 1 ml 5 M NaOH. The solution was saturated with NaCl and extracted with 10 ml diethyl ether by vortex mixing for 30 sec. The two phases were separated by centrifugation at 1500 g for 5 min and 0.5 ml of the organic layer transferred to a 1 ml vial, 0.05 ml triethylamine added, and the contents reduced to dryness in a jet of nitrogen.

(c) Derivatization

Ethanolic KOH (0.01 ml) 1% (w/v) and 0.05 ml 2% (w/v) PFB in ethanol were added to the residue in the vials which were capped and heated at 75°C for 40 min in order to form

a pentafluorobenzyl ester. After cooling, excess reagent was blown off under a stream of nitrogen and 0.01 ml H_2O and 0.02 ml n-heptane added. The vials were briefly vortex mixed and centrifuged at 1000 g for 1 min. The heptane phase was then ready for examination.

(d) Analysis

Heptane phase (2 μl) was injected into a gas chromatograph-mass spectrometer (LKB 9000 S) assembly. A 3% OV-1 column was used isothermally at 200°C with a helium carrier gas flow of 50 ml min^{-1}. PAA and its deuterated counterpart were detected by monitoring the ions m/e 316 and m/e 323 respectively. The analysis was carried out at 50 eV electron beam energy.

Every batch analysed contained a reagent blank in which an equal volume of H_2O was taken through the procedure.

Quantification was achieved by calculating the ratio of peak heights of PAA to D_7 PAA in the samples and referring these values to a calibration curve constructed freshly with each batch.

The correlation coefficient for the standard curve was never less than 0.998 (5 data points).

(v) DA method

PFP - Pierce Chemical Co., Rockford, U.S.A.;
α-hexachlorocyclohexane (α-lindane) - Koch-Light Labs Ltd., Colnbrook, U.K.;
alumina (neutral 100-240 mesh) - Hopkin and Williams Ltd., Chadwell Heath, U.K.;
OV-3 - Applied Science Labs, State College, U.S.A.

The assay employed was a modification of that of Wong et al (1973).

(a) Extraction

The crucial step in this method is the absorption of DA on to alumina columns. Before use, the alumina purchased was treated according to Weil-Malherbe (1961). This manoeuvre involves acid washing the alumina, drying and then removing the fines.

To 5 ml urine was added 0.2 ml 10% (w/v) EDTA, 0.2 ml 10% (w/v) ascorbic acid and 200 mg alumina. The pH of this mixture was raised to 8.6 with successive additions of 2 M, 1 M and 0.1 M NaOH whilst being mechanically stirred. It was then applied to glass columns plugged with cotton wool, containing 200 mg alumina which had previously been washed with 3 ml sodium acetate reagent. This reagent was made up of 250 ml 1.5% (w/v) sodium acetate, 5 ml 10% (w/v) ascorbic acid and 5 ml 10% (w/v) EDTA and was adjusted to pH 8.6. When the samples had drained through the columns, they were washed with 5 ml sodium acetate reagent followed by 5 ml H_2O. Finally, DA was eluted from the alumina by 3 ml 0.5 M acetic acid.

(b) Derivatization

Column eluate (0.2 ml) was freeze dried in a 1 ml screw-cap vial, 0.1 ml PFP was added, the vial capped and heated at 65°C for 1 h.

Just prior to analysis, excess PFP was evapourated under a stream of nitrogen. The residue was reconstituted in 0.1 ml n-heptane containing 0.25 μg ml^{-1} α-lindane as an injection standard.

(c) Analysis

1 μl was injected on to a 12 ft 10% OV-3 column in a Hewlett-Packard 5713A gas chromatograph equipped with a ^{63}Ni electron-capture detector. The oven temperature was kept at 210°C, whilst the injection port and detector were maintained at 300°C and 350°C respectively. Methane (5%) in argon carrier gas was passed at a rate of 30 ml min^{-1}.

Quantification was achieved by adding a known amount of DA to duplicate urine samples and carrying these specimens through the entire procedure. The peak height of DA relative to α-lindane was calculated in all samples, so that, by subtraction, the contribution to the peak height of the known amount of DA was worked out. Hence the quantity of endogenous DA in the "unspiked" samples could be ascertained.

Each batch of samples analysed contained a reagent blank

in which 5 ml H_2O was taken through the whole procedure.

(vi) Protein estimation

The method of Lowry et al (1951) was used. This colourimetric assay relies on two reactions: (a) that of protein with copper in alkaline solution and (b) reduction of phosphomolybdic-phosphotungstic reagent (Folin-Ciocalteu phenol reagent) by copper-treated protein.

The reagents used (Lowry's notation) were: solution A, 2% Na_2CO_3 in 0.1 M NaOH; solution B, 0.5% $CuSO_4.5H_2O$ in 1% (w/v) sodium tartrate; solution C, 50 ml of solution A plus 1 ml solution B; solution E, diluted Folin-Ciocalteu phenol reagent.

Enzyme mixture (0.02 ml) was diluted to 0.2 ml with H_2O. Solution C (1 ml) was added, mixed and left at room temperature for 10 min. Solution E (0.1 ml) was then added and the solution mixed immediately. After 30 min at room temperature, the optical density at 700 nm was read on a UV spectrometer (Pye-Unicam SP 1500).

A calibration curve was constructed using 0.02 ml of a diluted standard bovine albumin solution instead of enzyme. Samples were quantified by reference to this curve.

CHAPTER THREE - DEPRENYL AND THE "CHEESE EFFECT"

(i) The "cheese effect"

Even though MAO inhibitors were introduced into psychiatric medicine in the 1950s, it was not until 1963 that the first firm report (Blackwell, 1963b) appeared describing a hypertensive reaction in a patient receiving tranylcypromine. Soon afterwards cheese was identified as a precipitating factor (Blackwell, 1963c), and the TYR content of the food was quickly incriminated. (Asatoor et al, 1963).

The guises in which the syndrome can manifest were described by de Villiers (1966): either as a bad headache, or as a headache with cardiovascular involvement simulating the crisis observed in phaeochromocytoma, or most seriously as intracranial haemorrhage.

In retrospect, enough was known of the function of MAO, the TYR content of cheese and the pharmacological effects of TYR to have made it possible to predict that such dangerous side effects would occur. In 1913, Harley suggested that "end products of protein digestion may reach the general circulation if the quantity absorbed is larger than the liver can deal with." Somerville (1913) thought though that this could only occur if there were "some inability on the part of the oxidising machinery to keep pace with the formation of putrefactive products." Some years later Blaschko (1952) suggested that the MAO of the gut might function as a "detoxicating agent . . . if the food has been exposed to putrefaction and amines are already in it when eaten."

Van Slijke and Hart (1903) discovered that TYR is present in cheese, and a few years later the amine was observed to produce hypertension after oral administration (Dale and Dixon, 1909) and, in addition, to give rise to headaches following intramuscular injection (Findlay, 1911).

Following the report of Blackwell (1936c), many similar incidents were recognized and foods other than cheese were also shown to be involved, including cream, alcohol

(Bethune et al, 1964) chocolate (Krikler and Lewis, 1965), yeast extracts (Blackwell et al, 1965), pickled herrings (Nuessle et al, 1965) and chicken liver (Hedberg et al, 1966).

Chemical analysis of the foods triggering these reactions showed the presence of appreciable quantities of TYR (see Marley and Blackwell, 1970); but even this, and the identification of TYR metabolites in the urine after eating cheese (Asatoor et al, 1963), was only circumstantial evidence that TYR was the pressor substance responsible. Blackwell and Marley (1966) undertook a detailed investigation of the "cheese effect" in order to clarify its mechanism. They found that in rat and cat, intravenous injection of a cheese extract raises blood pressure, an effect potentiated by prior administration of a MAO inhibitor. However intraduodenally injected cheese produces no rise in blood pressure unless a MAO inhibitor has been given. After inhibitor treatment and intraduodenally administered cheese, a sympathomimetic amine was detected by bioassay in the circulation of the cat and, at the same time, a substance with the fluorescent characteristics of TYR was identified in the plasma. It had, therefore, been shown that there was a substance present in cheese capable of elevating the blood pressure of animals, which was only absorbed from the intestine into the systemic blood-stream after MAO inhibition. In an effort to confirm that the cheese pressor response was mediated by TYR they demonstrated that cheese and equivalent amounts of TYR had similar effects on the blood pressure of rats and cats and the nictitating membrane of the cat. In each instance, the response was halted by identical doses of an α-adrenergic receptor antagonist. Experiments involving chronic denervation of the nictitating membrane and the pharmacological tool, bretylium, indicated that this effect on α-receptors is mediated via indirect release of NA. Indeed this is the accepted mode of action of indirectly acting sympathomimetic amines such as TYR (Burn and Rand, 1958).

Thus there is considerable evidence that TYR is the pressor substance present in cheese. Indeed the oral TYR pressor

response in man (Horwitz et al, 1964) and in rat (Tedeschi and Fellows, 1964) is potentiated after MAO inhibition. However, it cannot be assumed that the adverse reactions are always due to TYR alone, as certain cheeses can contain PEA and tryptamine (Asatoor et al, 1963). Blackwell and Marley (1966) found that tryptamine only has a very small pressor response in animals when injected intraduodenally after MAO inhibition, as opposed to PEA which does have pronounced pressor activity after MAO inhibition. They also tested the potency of many amino acids, but showed that only dopa was active. Dopa does not commonly occur naturally; however Hoeldtke and Wurtman (1974) reported that a diet of certain cereals increases the excretion of its metabolites. Broad beans contain appreciable amounts of the amino acid, in fact their dopa content has been incriminated in an interaction with MAO inhibitors (Hodge et al, 1964). A parallel reaction has been observed in patients receiving a MAO inhibitor and L-dopa for the treatment of Parkinson's disease (Barbeau et al, 1962; McGeer et al, 1961; Hunter et al, 1970). Simultaneous administration of extracerebral decarboxylase inhibitors can reduce the likelihood of hypertensive episodes (Teychenne et al, 1975) but this combination was still considered too dangerous to use in the treatment of Parkinson's disease. The reduction of the problem by decarboxylase inhibitors indicates that it is probably dopamine formed from L-dopa which is responsible for the pressor action in this case.

(ii) Paradoxical absence of the "cheese effect"

A puzzling feature of the phenomenon is the ability of some subjects to ingest with impunity the particular food which has precipitated an attack in others (Sandler et al, 1975), or for a person occasionally to suffer a reaction after eating a certain substance or after ingesting a food-drug combination not previously known to be incompatible. Several explanations for this have been advanced. One of the most straightforward is that a moderate rise in systolic blood pressure produces severe head pain in some subjects but causes no concern to others (Blackwell et al, 1967). Alternatively, as TYR is absorbed from the caecum and duodenum rather than the stomach (Brodie and Hogben, 1957), factors which affect gastric emptying might

modify the "cheese effect."

Apart from the amines so far mentioned, some foods contain HA which relies substantially on histaminase for its inactivation (Chapter 7). As some MAO inhibitors affect histaminase (Shore and Cohn, 1960), their ingestion together with foods containing TYR and HA (e.g. yeast extracts) might lead to high circulatory levels of both. As they have opposite effects on blood pressure in man, they may tend to cancel each other out (Blackwell et al, 1965). In the light of this observation, it is interesting to note the report of Horwitz et al (1964) of a patient in whom cheese evoked a headache without producing hypertention and that of Senanayake et al (1978) where a hypotensive crisis occurred in a patient taking isoniazid (which inhibits histamase) after eating skipjack, a fish with a particularly high tissue content of HA.

Another factor which may cause variable responses to cheese consumption under conditions of MAO inhibition is the wide range of TYR concentrations even with a particular type of cheese. The content of 14 cheddar cheeses was found to range from 72 to 953 mg per g of cheese (Blackwell and Mabbit, 1965). Even within a particular sample, the TYR content may vary between the centre and the rind of the cheese (Price and Smith, 1971).

Variation in susceptibility to the "cheese effect," in at least one case (Marley and Blackwell, 1970), has been attributed to the recovery of the oxidising ability of the intestine on days when the inhibitor was not taken. However, it should be noted that Levine and Sjoersdma (1963) found that after MAO inhibitor treatment, human jejunal enzyme activity requires 8 or 9 days to recover fully. One other modifying factor was observed by Blackwell and Marley (1966). Some MAO inhibitors, such as tranylcypromine, have intrinsic sympathomimetic properties and shortly after a large dose of the inhibitor, tachyphylaxis readily develops to TYR. However, this wanes quickly and sympathomimetic changes in response to TYR are again seen if sufficient time is allowed to elapse.

In a proportion of patients who suffer headaches and hypertensive crises whilst taking MAO inhibitors, no interaction

with foodstuffs can be identified (Cooper et al, 1964). It is conceivable that this occurs because the intrinsic sympathomimetic effects of some inhibitors are enhanced once they themselves have caused sufficient inhibition of MAO. This aspect was investigated by Marley and Blackwell (1970) who found that only tranylcypromine and phenelzine are liable to "autopotentiation" to any significant degree. It is interesting to note, therefore, that these two inhibitors together are responsible for about 90% of hypertensive episodes in man (Marks, 1965).

(iii) Selective MAO inhibitors and the "cheese effect"

One aspect of the reaction which is not disputed is that, without MAO inhibition, the pressor substance in foods is not absorbed. With the synthesis of a selective MAO-A inhibitor, clorgyline, the possibility arose that TYR can be safely oxidised by one form of the enzyme whilst the other remains inhibited. Lader et al (1970), however, found that clorgyline is no less likely to provoke hypertension after amine ingestion than any of the conventional inhibitors. In fact, as little as 6.25 mg of TYR induced a reaction in one subject after receiving clorgyline for 6 days, whereas a cumulative dose of 750 mg TYR was required before MAO inhibition. The work of Squires (1972) provides an explanation for this. In a small number of samples investigated, he found that intestinal mucosa predominantly contains the A form of MAO so that, under conditions of MAO-A inhibition, TYR will by-pass the intestinal barrier and gain access to the circulation. If this reasoning is sound, then appropriate dosage of selective MAO-B inhibitors should be safe. Even before the selective MAO-B inhibitory properties of deprenyl had been really explored, Knoll et al (1968) reported that it possessed the peculiar property of inhibiting the pressor effects of TYR in animals.

As the evidence that deprenyl would not cause the "cheese effect" in man was tenuous, resting on the absence of complaints by patients receiving the drug in early trials for depression (Varga and Tringer, 1967), it was decided to test the effect of acute and chronic deprenyl treatment on the response to TYR and PEA loads in man. In addition, *in vitro* work on the MAO of

human intestinal biopsy material was carried out to check and extend the findings of Squires (1972) which are so crucial to the investigation of the "cheese effect."

In vivo biochemical measurements in man after MAO inhibition have been surprisingly lacking during the 30 years in which this class of drugs has been used. Those which have been carried out, however, have established that tissue enzyme activity is largely suppressed by clinical dosages. The drugs have relatively little effect on the urinary excretion of amines which have alternative routes of metabolism compared with those which rely solely on MAO (Sjoerdsma et al, 1959a; Levine and Sjoerdsma, 1963; Murphy et al, 1977). Because of the realtive paucity of information, the opportunity was taken to collect blood and urine samples in order to examine whether deprenyl changes PEA, PAA and DA concentrations and MAO activity. Samples were obtained before, during and after volunteers received deprenyl both during the study on the "cheese effect" (described below) and in another trial on the effect of deprenyl on sleep. After the amine loads had been administered, urine was also collected to investigate their handling after MAO inhibition.

Tryptamine output was formerly used as an index of MAO inhibition in man (Sjoerdsma et al, 1959b; Levine and Sjoerdsma, 1963). Concomitant measurements of platelet MAO activity and urinary PEA output after deprenyl were made in order to assess whether the latter also mirrors the MAO status of the body.

Methods

(i) Oral amine pressor tests

This study was passed by the University College Hospital and Medical School Committee on the Ethics of Clinical Investigations. The tests were performed in that hospital in the presence of an experienced physician with resuscitative measures at hand including a syringe loaded with phentolamine to be used if the blood pressure were to rise too high.

Four normal male volunteers and six parkinsonian patients (four male, two female) agreed to take part in the experiments. For several days prior to the first test, all subjects were

instructed not to ingest TYR or PEA-containing foods. After the baseline amine test, deprenyl administration was started, and the dosage was built up over the next week to 10 mg daily. The amine test was then repeated in all subjects, and yet again in four of the parkinsonians after eight weeks of continuous treatment with deprenyl.

The interval between TYR and PEA tests was at least three weeks in the case of the normal volunteers which meant that deprenyl was taken for two separate periods of one week. However, as the administration of deprenyl, once begun, was not terminated in the parkinsonian patients, the baseline TYR and PEA tests were both carried out before deprenyl treatment. After at least one week of drug ingestion the two amine tests were repeated. In both placebo and drug experiments, the TYR and PEA loads were separated by between two and seven days.

The tests were conducted in an unblind fashion in the manner described by Lader et al (1970). The subjects had fasted for about 12 h and took the daily dose of deprenyl 3 h before the start of the test. For the duration of the experiment they laid on a bed. After steady pulse rate and blood pressure readings had been recorded, the administration of the amine was begun. Progressively doubling doses were given at 30 to 45 min intervals, as solutions of amine hydrochloride in water. This commenced with 25 mg TYR hydrochloride or 6.5 mg PEA hydrochloride for the control tests, and 6.25 mg TYR hydrochloride or 0.65 mg PEA hydrochloride when subjects were receiving deprenyl. Blood pressure measured with a sphygmomanometer and pulse were recorded every five minutes. The criteria for terminating the tests were if the heart rate slowed by 10 beats per minute or when a systolic blood pressure rise of 20 mm mercury occurred, or if the doses reached 400 mg for TYR hydrochloride or 26 mg for PEA hydrochloride whilst the subjects were receiving deprenyl.

Blood and urine samples were collected for estimations of PEA and PAA concentration and MAO activity before, during

and after deprenyl treatment. The normal volunteers gave one 10 ml venous blood sample before the drug and three such samples on certain days whilst receiving deprenyl, at 2, 5 and 10 h after taking the drug; when treatment was stopped, single samples were drawn at the same time of day as the 5 h post-drug sample on days where this proved convenient. The parkinsonian patients only gave one blood sample on the day of each test. Three hour urine samples were collected on particular days from all subjects.

The methods used for these measurements are described in Chapter 2.

(ii) Urinary PEA, PAA and DA excretion before and after deprenyl

In a trial on the effect of deprenyl on sleep in man, 24 h urine collections were made on the third and final day of drug or placebo administration for the purpose of PEA estimation. DA and PAA were later also measured in these samples. Four subjects received 10 mg daily and two took 5 mg daily. Platelet MAO activity was determined on the same day as the urine samples were collected.

The assay procedures are described in Chapter 2.

(iii) Effect of deprenyl on intestinal and platelet MAO activity in vitro

Biopsy samples of human jejunal tissue obtained for diagnostic purposes and found to be normal were used for the experiments. After removal from the body, they were placed immediately on dry ice and then stored at -20°C until assay.

Blood collected from normal subjects was used; the harvesting of platelets and the MAO assay employed are described in Chapter 2.

(iv) Effect of deprenyl on the metabolism of orally ingested amines

Although the primary aim of the amine tests was to determine the safety of deprenyl, urine specimens were collected in addition during the mornings of the TYR and PEA loads, when

possible, to examine the metabolism of these compounds after MAO inhibition.

PEA was measured as described in Chapter 2. TYR was measured by Dr. S. Bonham Carter, employing the method described by Sandler et al (1975). Hydroxyphenylacetic acid was measured by Drs. C. Ruthven and B. Goodwin by the method of Goodwin et al (1976).

(v) Relationship between PEA excretion and MAO inhibition

In order to determine the degree of MAO inhibition required for a rise in urinary PEA to occur, small doses of deprenyl were administered daily to volunteers who then collected urine samples and gave blood for platelet MAO estimation each day. Baseline values were obtained from specimens collected before the drug was given.

The measurements were made using methods described in Chapter 2.

Results

(i) TYR pressor test

The effect of deprenyl on the TYR pressor response in normal volunteers is summarized in Table 3:1. The typical recordings for one subject are shown in Figure 3:1. Before the drug the subjects all reached a previously agreed maximum dose of 400 mg TYR hydrochloride (cumulatively 775 mg) before any rise in blood pressure or drop in pulse rate occurred. When these changes did occur, however, they were accompanied on occasion by sweating, pallor and a feeling of mild apprehension, but the subjects always reported noticing a forceful pulse in their head or chest at the height of the pressor response. After receiving deprenyl for a week, three of the four volunteers reached a dose of 200 mg (375 mg cumulatively), whilst the fourth continued to tolerate a dose of 400 mg TYR hydrochloride.

TYR tolerance before and after deprenyl administration to Parkinsonian patients is shown in Table 3:2. Their response was similar to that of normal subjects, although the

Table 3:1 To show response of healthy normal volunteers to oral TYR

Volunteer	Dosage of TYR hydrochloride at which response occurred (mg)		Pulse fall (beats/min)	Blood pressure rise (systolic/diastolic) (mm Hg)
1 M	Before deprenyl	400	10	28/0
	On 10 mg deprenyl	200	14	31/0
2 M	Before deprenyl	400	0	30/8
	On 10 mg deprenyl	400	0	22/6
3 M	Before deprenyl	400	20	40/3
	On 10 mg deprenyl	200	11	22/0
4 M	Before deprenyl	400	12	25/5
	On 10 mg deprenyl	200	16	20/10

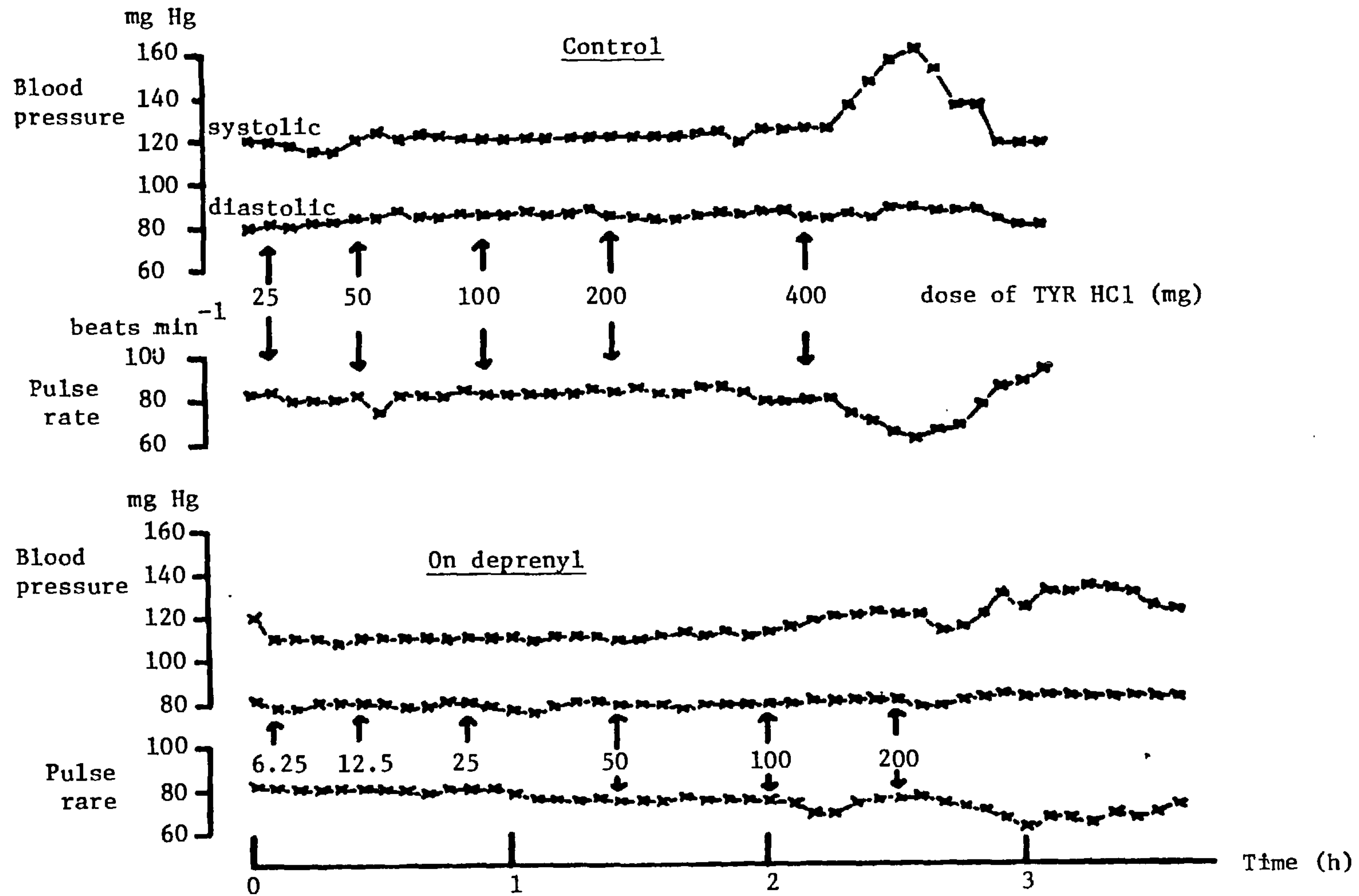


Fig. 3:1 Effect of deprenyl on the pressor response to oral TYR

Table 3:2 To show response of patients with Parkinson's disease to oral TYR

Patient (age)	Antiparkinsonian treatment (daily dose)	Dosage of TYR hydrochloride at which response occurred (mg)		Pulse fall (beats/min)	Blood pressure rise (systolic/diastolic) (mm Hg)
1 M (52)	None	Before deprenyl	400	0	30/5
		1 week 10mg deprenyl	200	0	25/0
		8 weeks 10mg deprenyl	200	0	30/5
2 F (72)	None	Before deprenyl	200	10	15/5
		1 week 10mg deprenyl	200	12	10/0
3 F (71)	Levodopa (3.5 g)	Before deprenyl	200	0	20/8
		1 week 10mg deprenyl	400	0	5/0
		8 weeks 10mg deprenyl	200	0	10/5
4 M (38)	Levodopa (3.0 g)	Before deprenyl	400	6	30/10
		1 week 10mg deprenyl	200	0	40/5
5 M (54)	Levodopa plus carbidopa (500 mg)	Before deprenyl	400	12	40/5
		1 week 10mg deprenyl	400	15	35/5
		8 weeks 10mg deprenyl	400	21	50/5
6 M (62)	Levodopa plus carbidopa (900 mg)	Before deprenyl	400	12	45/15
		1 week 10mg deprenyl	150	20	50/20
		8 weeks 10mg deprenyl	150	24	45/15

lability of blood pressure in L-dopa treated patients made assessment difficult, as did the absence of bradycardia in some subjects. There was no alteration in TYR tolerance after eight weeks of continuous daily deprenyl treatment.

(ii) PEA pressor test

The only effect of PEA administration before deprenyl in the normal volunteers was a delayed amphetamine-like reaction in one of the subjects, occurring about 45 min after taking the final (130 mg) dose of PEA hydrochloride (245 mg cumulatively). This response consisted of tachycardia (120 beats per minute), ectopic pulse beats, dilated pupils and feelings of mild apprehension and increased energy.

One parkinsonian developed a pressor response to 65 mg PEA hydrochloride which was rapidly reversed by 5 mg phentolamine intravenously.

However, no adverse effects occurred in any of the normal volunteers whilst taking deprenyl and PEA in doses of up to 20 mg, or in any of the parkinsonian patients who all received 26 mg of the amine hydrochloride.

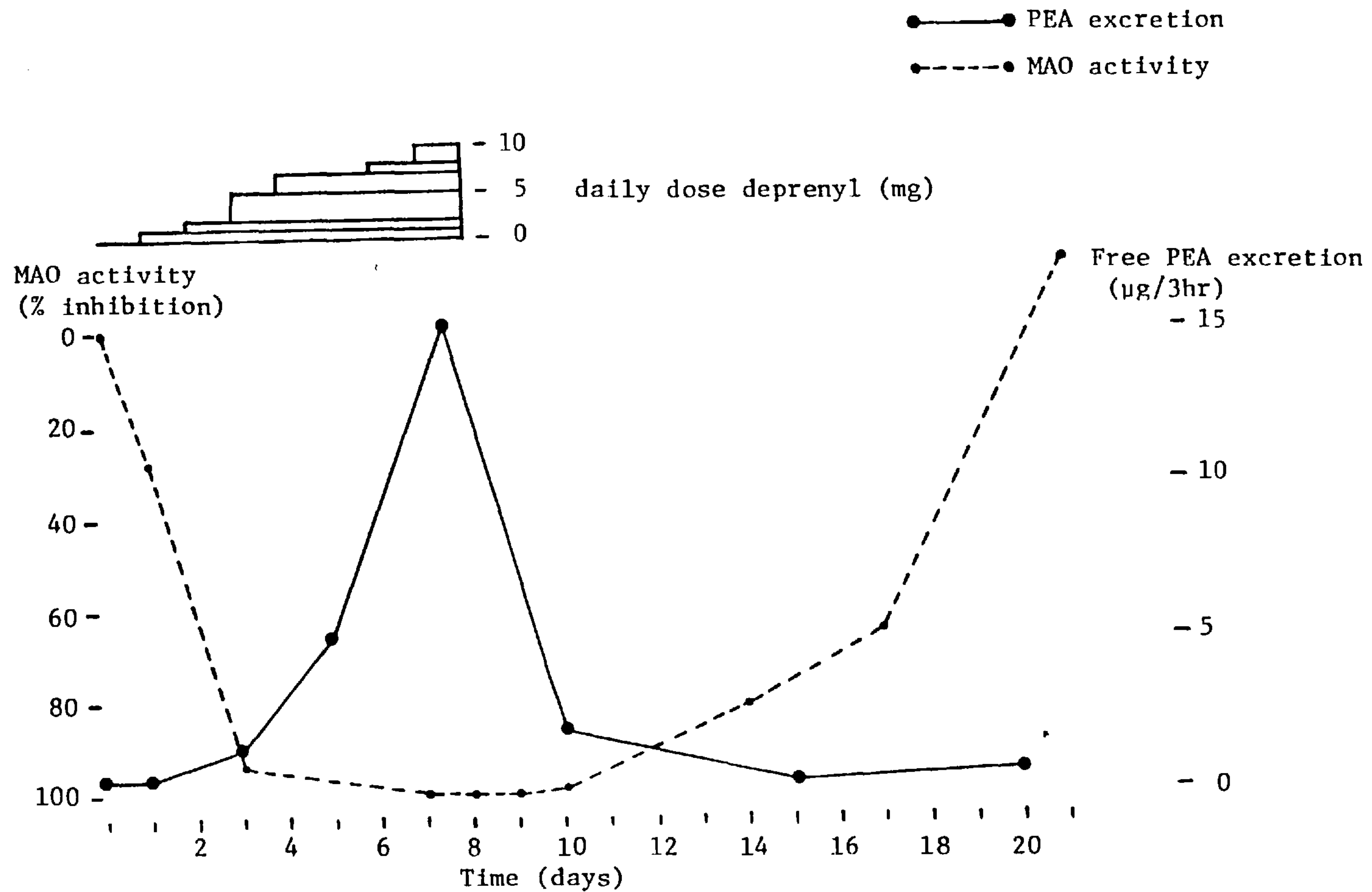
(iii) Inhibition of MAO by deprenyl

(a) in vivo

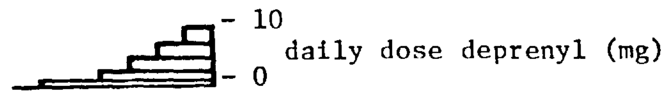
The effect of deprenyl on platelet MAO from the normal volunteers who took part in the amine loading experiments are shown in Figures 3:2 to 3:5. In all four, and also in the patients participating in the tests, platelet MAO activity was absent at the time of the pressor tests. In three of the normal subjects, enzyme activity was reduced by 20% or more 2 h after receiving 1 mg deprenyl. Although the recovery rate varied between subjects, enzyme activity was consistently absent the day after cessation of deprenyl administration.

(b) in vitro

Figure 3:6 shows the inhibitory effect of deprenyl on PEA oxidation by platelet MAO in comparison with the suppression of TYR oxidation by intestinal mucosa. The platelet enzyme is about one thousand times more sensitive to deprenyl than the intestine.



<u>Fig 3:2</u> Showing changes in platelet MAO activity and PEA excretion in volunteer 1 receiving deprenyl.



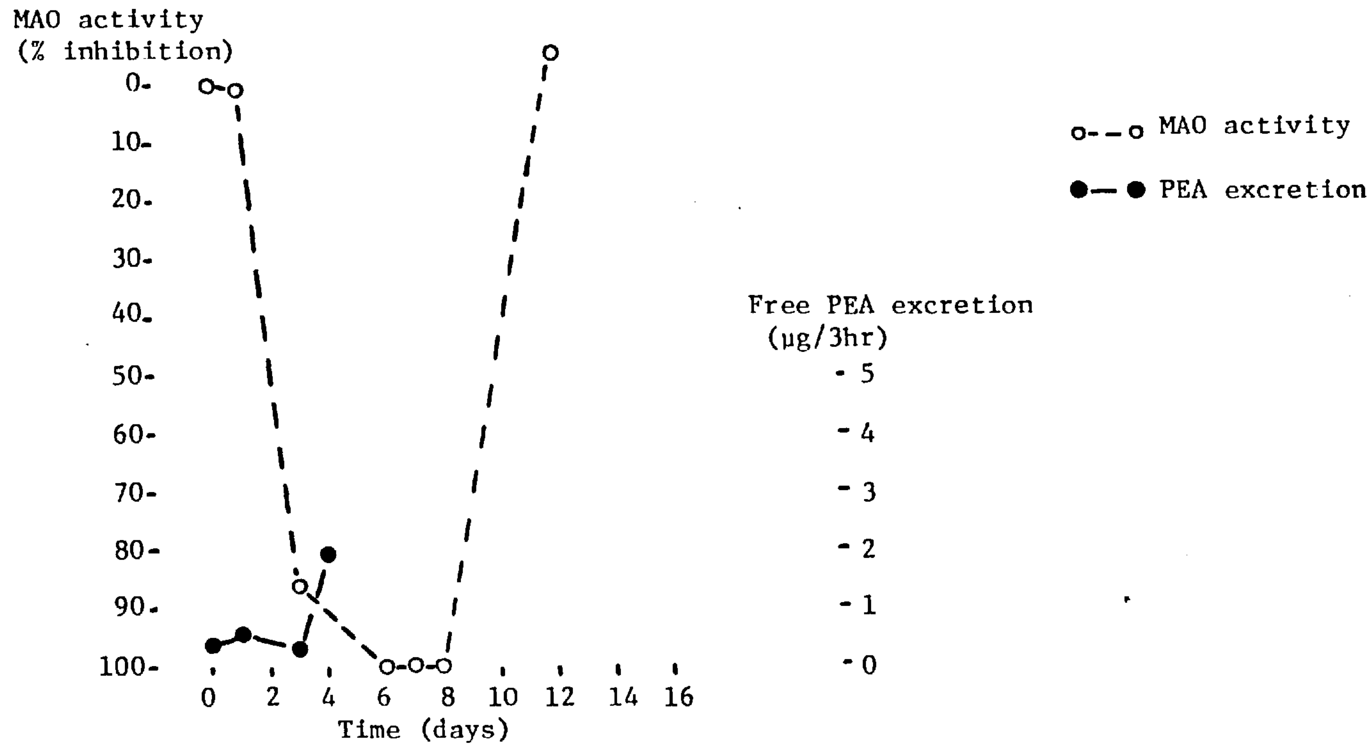


Fig. 3:3 Showing changes in platelet MAO activity and PEA excretion in volunteer 2 receiving deprenyl.

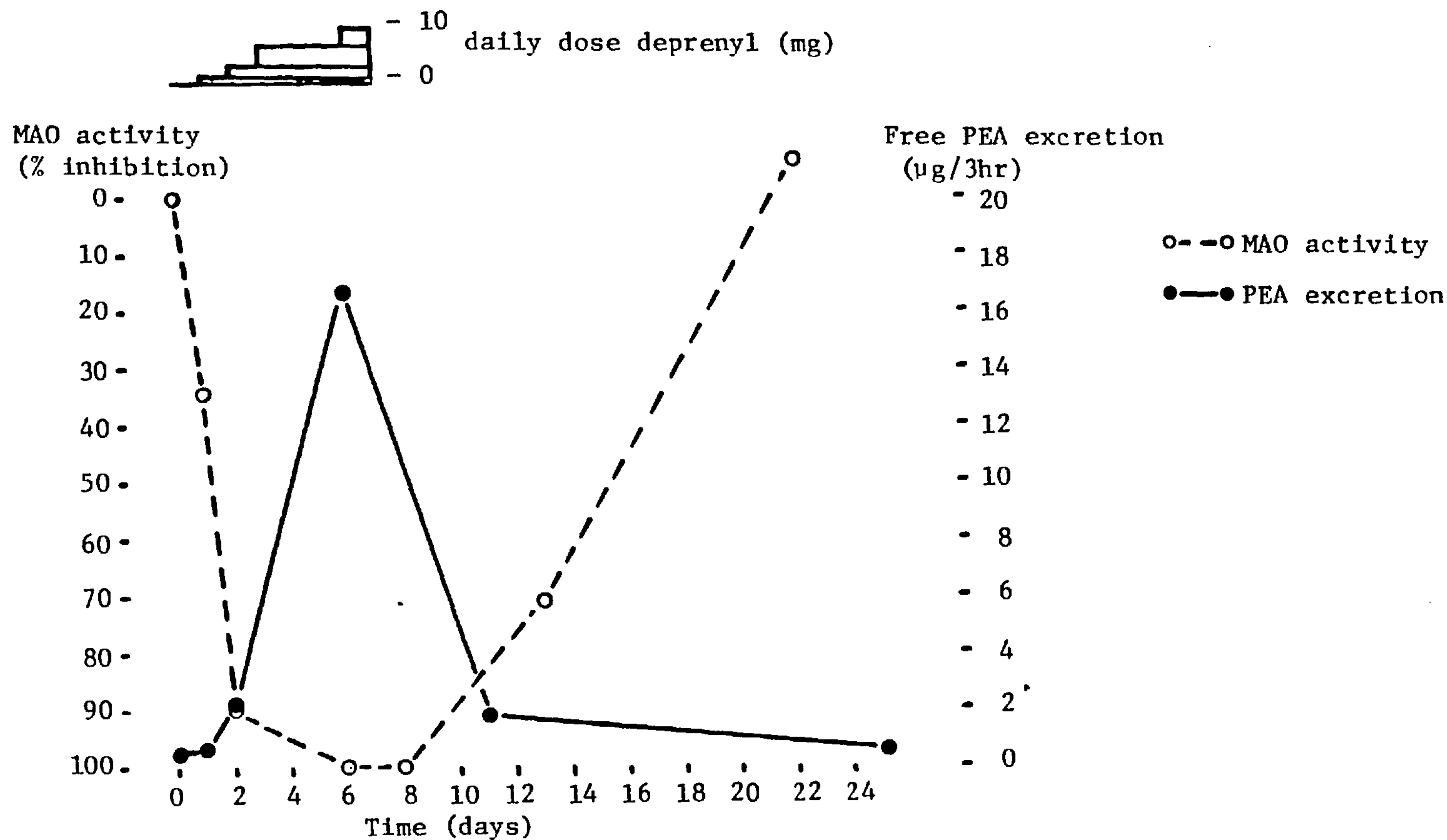


Fig. 3:4 Showing changes in platelet MAO activity and PEA excretion in volunteer 3 receiving deprenyl

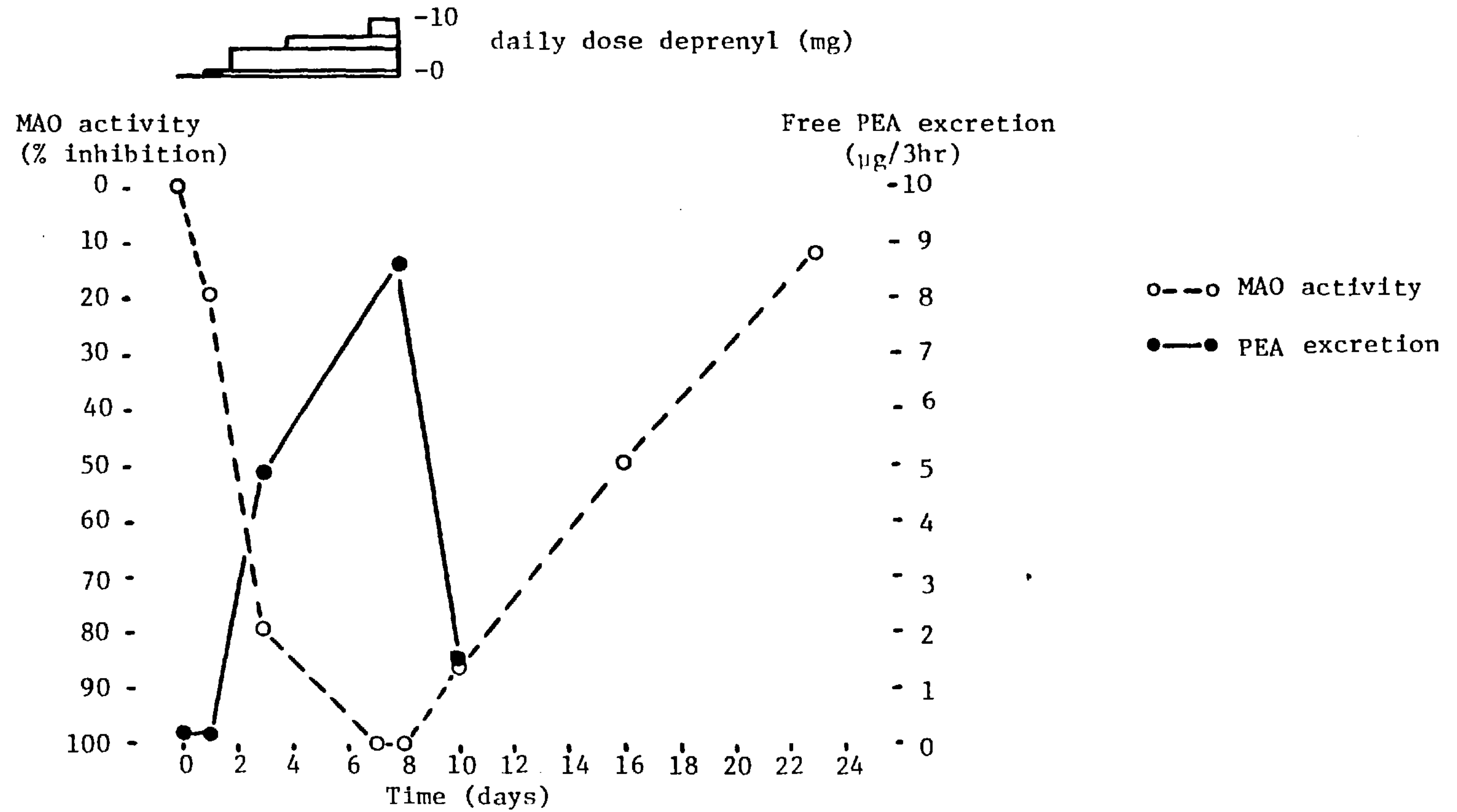


Fig. 3:5 Showing changes in platelet MAO activity and excretion in volunteer 4 receiving deprenyl

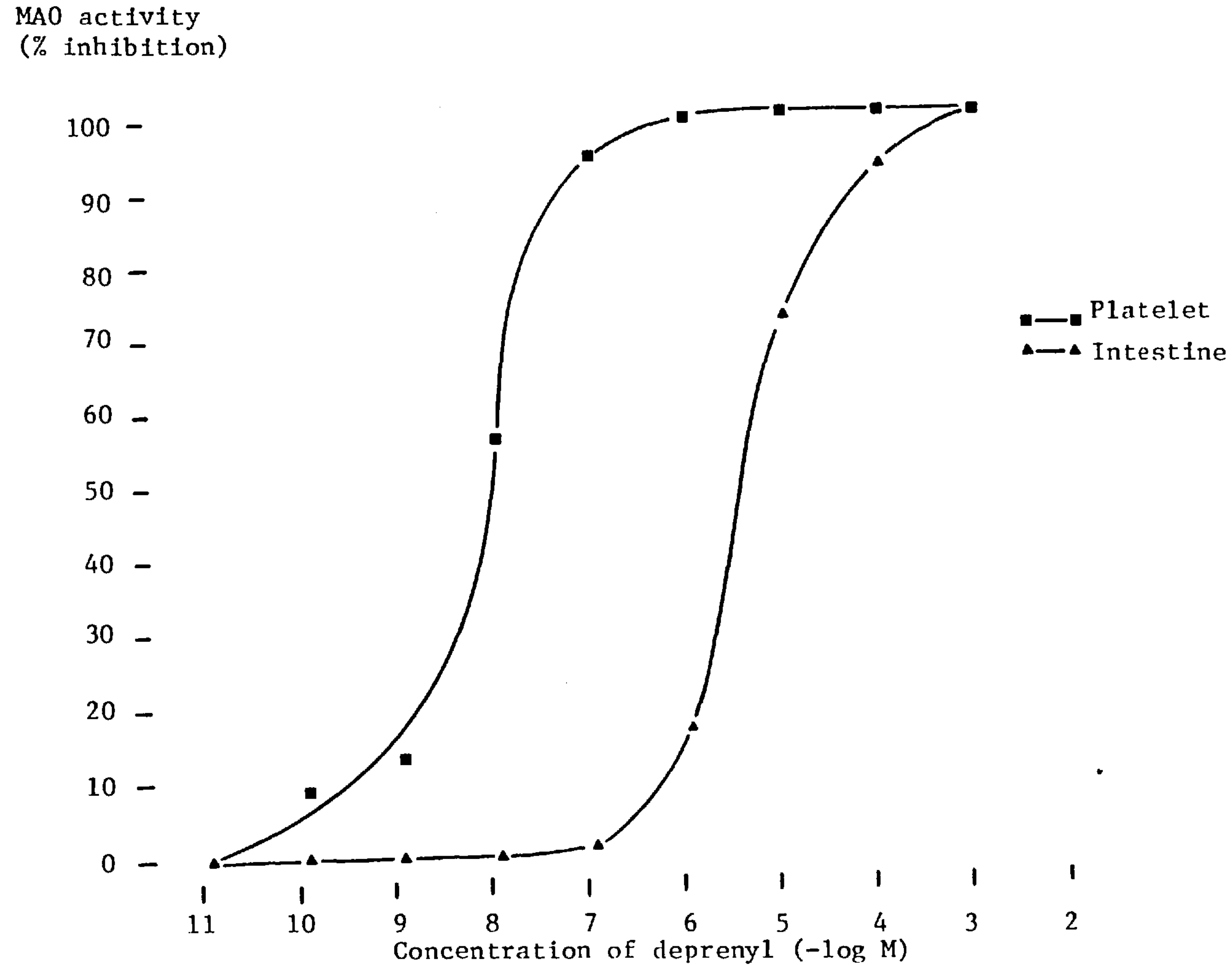


Fig. 3:6 *In vitro* deprenyl inhibition of intestine and platelet MAO

TYR (150μM) oxidation by intestinal mucosa, each point represents mean of 3 samples, and PEA oxidation by platelet (25μM), each point represents mean of 2 samples.

(iv) Deprenyl and PEA excretion

(a) Effect on free amine

The free PEA output in the normal volunteers taking part in the amine pressor tests is shown together with their platelet MAO status in Figures 3:2 to 3:5. The excretion was not altered by 1 mg deprenyl but did rise as the dose was increased and the MAO inhibition approached 100%. At the time of the amine pressor tests the volunteers had a urinary concentration raised up to 90 times above baseline values. After withdrawal of the drug, the return to normal concentration occurred more rapidly than the reestablishment of the platelet MAO activity.

The urinary PEA concentration determined in the 24 h urine collection from normal volunteers before and during deprenyl treatment is shown is Table 3:3. Platelet MAO inhibition was complete or almost total in all cases, and the rise in PEA excretion was statistically significant ($p<0.01$, paired t-test).

The normal range for the rate of PEA excretion was calculated from analysis of urine collections from 19 normal subjects (Table 3:4). The mean value ± SEM was 4.99±0.44 μg PEA per 24 h.

(b) Effect on the conjugated amine

Although deprenyl causes a clear-cut elevation in free urinary PEA concentration, the increase in the output of the conjugated form is less consistent (Table 3:5).

(v) Relationship between MAO inhibition and PEA excretion

Table 3:6 lists the highest degree of inhibition of platelet MAO compatible with normal PEA excretion, together with the inhibition produced by the following dose of deprenyl which raised the PEA output. The study shows that 79% inhibition of platelet MAO was the lowest which corresponded to a raised PEA concentration, and 86% was the most inhibited the platelet enzyme became without concomitant rise in PEA excretion.

(vi) TYR metabolism after MAO inhibition

These urine analyses provided no meaningful data, as subsequent to the analysis it was found that hydroxy-amphetamine, a likely metabolite of deprenyl (Chapter 6), interferes with TYR in the assay procedure.

Table 3:3 Effect of deprenyl on PEA excretion in normals

Subject	Baseline PEA excretion (μg/24hr)	PEA excretion on deprenyl (μg/24hr)	Deprenyl dose (mg/day)	% Platelet MAO inhibition
1	4.32	86.08	5	100
2	4.22	24.32	5	97
3	4.04	56.88	10	100
4	4.21	39.10	10	100
5	7.29	25.36	10	79
6	7.71	18.29	10	98

Table 3:4

Showing the variation in free PEA excretion in normals*

Subject	PEA excretion (μg/24hr)	Subject	PEA excretion (μg/24hr)
1	3.27	11	4.22
2	3.29	12	4.32
3	3.51	13	4.93
4	3.59	14	5.82
5	3.64	15	6.13
6	3.64	16	7.22
7	3.72	17	7.29
8	4.00	18	7.71
9	4.04	19	10.23
10	4.21		

mean = 4.99 ± 0.44 (S.E.M.)

* Listed in order of magnitude

Table 3:5

Comparing the effect of deprenyl on the excretion on free and conjugated PEA

Subject	Free PEA (μg/24hr)		Conjugated PEA (μg/24hr)	
	Baseline	Deprenyl	Baseline	Deprenyl
1	3.72	50.11	24.34	43.16
2	4.93	45.94	24.80	77.49
3	10.23	32.00	22.94	21.66

Table 3:6

Relationship between platelet MAO activity and PEA excretion

Subject	% Platelet MAO inhibition before PEA excretion rose	% Platelet MAO inhibition at which PEA was elevated
1	46	91
2	19	79
3	34	90
4	86	100
5	80	95
6	70	93
7	82	97
8	56	96
9	82	*
10	85	*

*Deprenyl administration ceased before any change in PEA excretion occurred.

(vii) PEA metabolism after MAO inhibition

The total PEA output (free and conjugated) in three normal volunteers receiving a load of PEA before and during deprenyl administration is shown in Table 3:7.

These collections contained a higher output of PEA than is normally seen during deprenyl treatment (Table 3:3). The extra amount excreted is difficult to quantify but probably represents, at the most, 1% of the ingested dose.

The oxidatively deaminated metabolites of the tyramines were also measured in these urine specimens (Table 3:7). Although there did appear to be a rise in the excretion of these compounds after deprenyl, it was not sufficient to account for the total load of PEA.

(viii) Effect of deprenyl on PAA

(a) Plasma concentration

Deprenyl (10 mg daily) did not alter the plasma free PAA concentration in normal subjects (Table 3:8).

(b) Urinary concentration

Neither free nor conjugated excretion values of PAA (Table 3:9) were changed by deprenyl treatment.

(ix) Effect of deprenyl on DA excretion

The output of free DA was not altered by deprenyl (Table 3:10).

Discussion

The amine pressor tests showed unequivocally that deprenyl, unlike clorgyline (Lader et al, 1970), does not give rise to the "cheese effect" (Table 3:1 and 3:2). The volunteers were all able to ingest amounts of TYR and PEA in excess of that likely to be encountered in a normal diet. TYR is the principal pressor substance involved in the "cheese effect," and the greatest concentration in which it has ever been recorded is 1.4 mg per g in New York State Cheddar Cheese (Horwitz et al, 1964). Even the subject who tolerated TYR the least would have been able to eat about half a pound of this variety of cheese.

It is especially interesting that the two parkinsonian patients treated with an L-dopa-deprenyl combination did not experience any untoward pressor effects (Table 3:2), although

Table 3:7

Effect of deprenyl on PEA and hydroxylphenylacetic acid (HPA) excretion after PEA ingestion

Subject	Deprenyl dose (mg/day)	Total dose of PEA ingested (mg)	Collection* period (hrs)	Total** PEA excretion (μg)	Ortho-HPA excretion (μg)	Meta-HPA excretion (μg)	Para-HPA excretion (μg)
1	0	50.5	0-3	5.28	91	275	713
			3-6	5.50	29	100	318
	10	6.5	0-3	34.90	133	818	1775
			3-6	45.51	30	206	363
2	0	50.5	0-3	3.55	85	26	79
	10	11.5	0-3	14.39	112	86	63
			3-6	31.88	55	80	60
3	0	187.5	0-3	0.49	23	16	113
	10	20.0	0-4	7.08	150	67	250
			4-8	62.72	48	148	1270

*from beginning of experiment

**total excretion is free plus conjugated excretion

Table 3:8

Effect of deprenyl on plasma PAA concentration

Subject	Plasma PAA (ng/ml)	
	Baseline	on 10 mg deprenyl daily
1	13.4	19.0
2	150.8	83.2
3	56.6	42.1
4	262.2	344.4
5	84.8	92.2

N.S.D.

Table 3:9

Effect of deprenyl on urinary PAA concentration

Subject	Free PAA (μg/24 hr)		Conjugated PAA (mg/24hr)	
	Baseline	Deprenyl	Baseline	Deprenyl
1	276	250	59.9	40.7
2	398	437	53.4	64.1
3	220	362	84.0	*
4	490	226	90.8	53.4
5	375	406	88.8	127.9
6	456	442	170.9	123.5
	N.S.D.		N.S.D.	

*sample lost

Table 3:10

Effect of deprenyl on urinary DA excretion

Subject	DA excretion (μg/24hr)	
	Baseline	Deprenyl
1	271	194
2	174	261
3	307	154
4	178	180
5	247	193
6	166	241

N.S.D.

they occur when conventional inhibitors are used. Although DA is metabolized by the MAO-B of the platelet, it can also be degraded by MAO-A in the intestine (Chapter 5). It is possible, therefore, that DA generated in the gut is important for the hypertensive adverse reaction if it is not also inactivated there. Alternatively, other peripheral tissues possessing DA oxidising MAO-A activity may also provide a safeguard.

Deprenyl only discriminates between the different forms of MAO within a certain concentration range (Figure 3:6). Maximum selectivity is achieved at about 10^{-6}M deprenyl, the concentration the usual daily dose, 10 mg, would produce if it were evenly distributed in an average human water space of 40 kg. Whilst it is not disputed that this concentration is selective in vitro, several workers have been concerned that this is not the case in vivo. Egashira et al (1976) noted that even prolonged incubation of either deprenyl or clorgyline with the enzyme in vitro results in a loss of selectivity of inhibition. Waldmeier and Felner (1978) reported that chronic administration of deprenyl in the rat reduced its selectivity; however, a rather high dose of deprenyl, about 10^{-5}M was used. Much smaller doses were employed by Knoll (1978) who found that at about 10^{-6}M, deprenyl selectively inhibits rat brain MAO for up to 3 weeks. However, in the field of MAO studies it has already been shown that one has to be very cautious in extrapolating from one species to another (Glover et al, 1977). The data concerning the selectivity of deprenyl in man are unfortunately rather inconclusive. Studies on MAO in post-mortem brains from parkinsonian subjects who had received deprenyl for an average of 6 days prior to death showed that oxidation of DA (predominantly a B substrate, Chapter 5) was inhibited by 82-88%, whereas 5HT oxidation was inhibited by 50-75% (Riederer et al, 1978). The latest data from this group indicate that these levels of inhibition do not then increase further with prolonged periods of treatment (Riederer and Reynolds, 1980). However, the answer to the selectivity question can only come from measurements of actual amine substrates or their metabolites, for we do not know whether the reduction in MAO-A activity observed by Riederer and his group is sufficient to result in an accumulation of MAO-A

substrates. Table 3:6 indicates that about 80% of MAO-B activity needs to be inhibited before the concentration of PEA builds up. Robinson et al (1973) and Ravaris et al (1976) found that 80% inhibition of platelet MAO was necessary before phenelzine became superior to placebo in the treatment of depression. Davidson et al (1978), however, found that the drug could be effective when only giving rise to a 60% reduction in platelet MAO. In the rat, Green et al (1977) reported that an 85% inhibition of brain MAO was required before L-dopa produced behavioural alterations. It is evident, then, that a very large proportion of the enzyme activity needs to be inhibited before any functional alterations occur.

In an attempt to shed some light on the mechanism of the "cheese effect" Sandler et al (1978) performed some experiments in the pig. This animal was specifically chosen as in many of its tissues TYR oxidation is carried out predominantly by MAO-B (Ekstedt and Oreland, 1976). The pig gut is an exception, however; TYR is oxidised there by MAO-A. If freedom from the "cheese effect" in man results from an intact gastrointestinal MAO-A barrier, then by-passing it with intravenously administered TYR after deprenyl treatment should produce a hypertensive response, as MAO-B in the body would be inhibited. As it turned out, however, no such reaction was observed. Conversely, clorgyline, whilst only producing slight MAO inhibition, caused a hypertensive reaction. To interpret these findings, the authors proposed that two separate pharmacological effects are normally found with MAO inhibitors, enzyme inhibition and facilitation of NA release from its binding sites during TYR challenge: an absence of the second property is responsible for the relative safety of deprenyl in clinical practice.

In the light of this hypothesis, it is interesting to note that isoniazid, which does not inhibit MAO, has been responsible for producing the "cheese effect" (Robinson et al, 1968; Smith and Durack, 1978). A similar reaction has also been described following treatment with indomethacin (Lee et al, 1974) which is also devoid of MAO inhibitory properties.

The absence of "cheese effect" with deprenyl has been explained in other ways. Knoll and Magyar (1972) showed that

deprenyl, albeit in high dosage, inhibits amine uptake mechanisms and also prevents the outflow of NA from its storage sites. They considered that these factors are responsible for the absence of the "cheese effect." Knoll (1976) widened this explanation to include the evidence of Squires (1972) suggesting that deprenyl would preserve the intestinal MAO-A barrier intact.

The metabolism of deprenyl should also be taken into account when considering the mode of action the drug. It has been found to be converted almost quantitatively to metamphetamine and amphetamine (Chapter 6). Although it seems likely that the less active (-)-isomers are generated, it is nevertheless interesting to record that Cavanaugh et al (1970) reported that chronic administration of (+)-amphetamine diminishes the pressor response to TYR in man.

It is clear from the preceeding discussion that the precise mechanism responsible for the absence of the "cheese effect" is not yet resolved. It seems likely though, that deprenyl differs from conventional MAO inhibitors in a way other than selectivity of inhibition, and that this other effect, probably on egress or amine uptake, in addition to an MAO-A sparing capacity of deprenyl, will explain this important feature of the drug. Whatever the mode of action, it is important to note that freedom from the "cheese effect" is not a transient phenomenon (Table 3:2). Indeed it appears that TYR tolerance is not altered even after 18 months continuous daily treatment with deprenyl (Stern et al, 1978).

An interesting outcome of the biochemical studies, already mentioned, is that PEA excretion rose sharply when about 80% inhibition of the platelet enzyme had been achieved (Table 3:6). Although the urinary excretion of PEA is somewhat pH dependent (Reynolds et al, 1978), the magnitude of the output is such that it overcomes this effect so that urinary PEA concentration appears to be a useful clinical index of _in vivo_ MAO-B inhibition, just as urinary tryptamine was used as a guide to overall inhibition of the enzyme (Sjoerdsma et al, 1959b; Levine and Sjoerdsma, 1963). Gottfries and Magnusson (1962) found that between 3- and 19-fold increases in the urinary concentration of tryptamine occurs as a result of MAO inhibitor administration

in man. More recently, Murphy et al (1977) reported that phenelzine treatment leading to an 82% reduction of platelet MAO activity is associated with an 8-fold elevation in urinary tryptamine output. These rises in tryptamine excretion are of a similar order to those in PEA observed after deprenyl treatment in the present study (Table 3:3) although, on occasion, the rise in PEA output was greater. The large variation normally found in excretion of conjugated PEA contrasts with the consistent output of the free form (Table 3:5; Reynolds and Gray, 1976) and renders measurements of the former an inaccurate index of MAO inhibition.

The determination of platelet MAO activity in this series of experiments revealed that the enzyme is extremely rapidly inhibited, in contrast with the response to some conventional inhibitors such as isocarboxazid (Robinson et al, 1968) and phenelzine (Murphy et al, 1977); this may conceivably be a dose effect. Because of the irreversible nature of the MAO inhibition caused by deprenyl, the return of activity after cessation of treatment took about 1 - 2 weeks. PEA excretion reestablished itself much more quickly, however, presumably because even a small amount of MAO activity can cope with the metabolism of the amine (Table 3:6). Even though there appeared to be some variation in recovery rate of MAO amongst the normal volunteers (Figure 3:2 to 3:5), measurements were not made with sufficient frequency to obtain an accurate assessment. One factor which should be kept in mind, however, is that platelet MAO activity was expressed in terms of milligrams of protein (as is usual) and not as a function of the number of platelets. This factor may be relevant as newly synthesized platelets tend to be larger, more dense and have greater MAO activity than older ones (Murphy et al, 1978).

The adverse reaction to oral PEA in one normal volunteer prior to deprenyl treatment was probably idiosyncratic. Larger doses of the amine have been safely ingested (Chen, 1927; Seakins, 1971). That the administration of PEA under conditions of MAO inhibition was without effect was initially surprising. Amphetamine-like effects are known to ensue in animals given this combination of drugs (Mantegazza and Riva, 1963). A probable explanation is that insufficient PEA was administered,

especially in view of the small amounts of free or conjugated PEA recovered in the urine after the load (Table 3:7). The fate of the ingested PEA was not discovered. As urine was not collected for longer than 8 h after the beginning of the PEA challenge, it is possible that unmetabolized amine was retained in the body and excreted after the cessation of urine collection. Alternatively, some might still conceivably have been converted to PAA either by traces of uninhibited MAO-B, or perhaps even by MAO-A. Other pathways of metabolism besides oxidative deamination and conjugation are known to exist for PEA. After MAO inhibition in the rat, a proportion of PEA is converted to octopamine and phenylethanolamine (Saavedra and Axelrod, 1973; Wu and Boulton, 1975), and also to o-, m-, and p-tyramine (Boulton et al, 1974). The absence of strikingly elevated levels of the acid metabolites of the tyramines (Table 3:7), is evidence against ring-hydroxylation of PEA being a major metabolic pathway. N-Methylation of PEA has also been suggested (Hsu and Mandell, 1973), and a Schiff base condensation between PEA and pyridoxal has been identified (Loo, 1967). Routes of metabolism which deserve further attention include PAA production from PEA via phenylpyruvate and the acetylation of PEA. That metabolism of PEA is not completely halted by *in vivo* MAO inhibition in man supports the work of Oates et al (1963) who reported that under these conditions, less than 10% of an infused dose of PEA is recovered unchanged in the urine in 12 h.

Although PAA is the major end product of PEA metabolism in the rat (Wu and Boulton, 1975), the absence of any effect of deprenyl on the free or conjugated PAA excretion (Table 3:9) is not necessarily incompatible with this. Indeed, deprenyl has been previously found to be without effect on rat urinary PAA concentration (Sandler et al, 1976), and this was attributed to the vast contribution of PAA from the gut flora; and subsequent investigations by this group on germ-free rats supported this claim.

The absence of any changes in plasma was more unexpected (Table 3:8) but one possible explanation for this finding, together with another reason for the presence of large amounts of the acid in the urine is that ingredients of many foodstuffs are converted

to PAA (Goodwin, 1976).

In view of our interest in deprenyl as an antiparkinsonian agent (Chapter 4), it was decided to measure the effect of the drug on urinary DA output in normals. No significant change was found (Table 3:10). Rinne et al (1978), however, did report a statistically significant increase in the excretion of DA in parkinsonians taking deprenyl with either L-dopa alone or in combination with a decarboxylase inhibitor, although this was based on only 2 and 3 patients respectively. Murphy et al (1977) found that MAO inhibition in depressed patients does not result in any significant changes in urinary excretion of the catecholamines, NA and A, presumably because of available alternative pathways of metabolism. DA, unlike the other catecholamines, however, is present in the urine in far greater quantities than can be explained on the basis of simple plasma clearance (Christensen, 1973) and it seems likely that free DA is actually added to the urine by the kidney (Ball et al, 1978) where it seems to be involved in the control of sodium excretion (Ball and Lee, 1977). These factors, therefore, probably explain the lack of effect of MAO inhibition on DA excretion.

In conclusion, a profound effect of deprenyl on PEA metabolism and MAO has been demonstrated and this, together with evidence that the drug is not associated with the "cheese effect," should ensure a continued role for deprenyl both as a research tool and in clinical practice.

CHAPTER FOUR - DEPRENYL IN PARKINSON'S DISEASE

The definitive description of this condition ("The Shaking Palsy") was made by James Parkinson (1817) who wrote a meticulous account of its classical signs, tremor, rigidity, and hypokinesia, together with disturbed posture and gait.

(i) Aetiology

In the majority of patients, the cause of the disease is idiopathic ("paralysis agitans"). However, several variants of known aetiology have been positively identified. An epidemic of the viral disease "encephalitis lethargica", occurred in the 1920s and affected individuals developed signs of so-called postencephalitic parkinsonism at varying intervals after the acute infection. Another variant the existence of which is to some extent debated, is arteriosclerotic parkinsonism, which characteristically affects the elderly: and neuronal degeneration in these instances stems from vascular changes. Parkinsonism is also a recognized side-effect of many major tranquillising drugs and this form is usually reversible. Ingestion of certain toxic materials has also induced a parkinsonian syndrome, e.g., manganese intoxication (Mena et al, 1967) and carbon monoxide poisoning (Garland and Pearce, 1967).

(ii) Pathology

The symptoms of idopathic Parkinson's disease appear typically between the ages of 50 and 60, and become progressively worse in the following years.

Degeneration of cells in the basal ganglia is characteristically, but not invariably, found in the parkinsonian brain (Stern, 1966). Pakkenberg and Brody (1965) reported a marked reduction in neuronal density in the substantia nigra of post-mortem brains from affected subjects, with the melanin containing cells being more depleted than the non-pigmented cells. A well defined pathway runs from the substantia nigra to the corpus striatum. This latter structure was found to contain a high concentration of DA, but only a relatively small amount of NA (Carlsson, 1959). This

discovery gave support to the suggestion of Blaschko (1957) that DA might possess regulatory functions of its own, apart from serving as a precursor for NA. Soon afterwards, Ehringer and Hornykiewicz (1960) reported a profound depletion of DA in the parkinsonian brain. This finding, together with the observation that reserpine, which depletes the brain of DA, produces parkinsonian-like symptoms (Carlsson et al, 1958) led to the suggestion that Parkinson's disease is a DA deficiency syndrome. The nigro-striatal pathway was specifically incriminated when appropriate brain lesions were shown to cause a substantial loss of DA containing cells in the substantia nigra (Andén et al, 1964) and of nerve endings in the corpus striatum (Poirier and Sourkes, 1965). During the intervening years, the hypothesis that the signs of parkinsonism arise from a DA deficiency in the striatum gained widespread acceptance (Hornykiewicz, 1973). However, other biochemical changes have been identified in Parkinson's disease, and in other parts of the brain, apart from the substantia nigra and corpus striatum. Alterations, for example, in the content of 5HT (Bernheimer et al, 1961), NA (Ehringer and Hornykiewicz, 1960), tyrosine hydroxylase, dopa decarboxylase (Lloyd et al, 1975) and glutamic acid decarboxylase (Hornykiewicz et al, 1976) have been reported in parkinsonism. The extent to which the disease can accurately be described as just a DA deficiency syndrome has recently been discussed by Sandler (1977).

(iii) Treatment

(a) L-Dopa

The discovery of the depleted DA content of the parkinsonian striatum led logically to the suggestion that restoration of the missing amine would reverse the clinical manifestations of the disease (Barbeau, 1962). As DA itself does not cross the blood-brain barrier, its immediate precursor, L-dopa, which does enter the brain after peripheral administration, was tested. First attempts with this form of treatment employed relatively low doses of L-dopa and were only moderately

successful (Birkmayer and Hornykiewicz, 1961; Barbeau, 1961). Using the racemic form of dopa in high dosage though, Cotzias et al (1967) obtained a much more striking benefit. Akinesia was relieved more consistently than rigidity or tremor. The idiopathic form of the disease responded more favourably to dopa treatment than the postencephalitic form (Krasner and Cornelius, 1970).

Very large doses needed to be given in order for sufficient to penetrate the brain, as much the greater proportion of the drug was metabolized peripherally (Bianchine et al, 1973). Side effects, due perhaps to large amounts of dopa metabolites, soon became apparent (Barbeau and McDowell, 1970). However, many of these problems were alleviated by the adoption of peripherally acting decarboxylase inhibitors. Administered along with L-dopa, they prevented its decarboxylation outside the brain, thus allowing smaller doses of L-dopa to be used to achieve a similar central effect (Birkmayer, 1969). The combination also reduced the likelihood of troublesome peripheral side effects (Krayenbühl and Siegfried, 1970).

(b) Other forms of treatment

Prior to the introduction of dopa therapy for Parkinson's disease in the 1960s, drugs had only been administered on an empirical basis. Ordenstein (1867) described the relief from muscular rigidity which could be derived from the administration of belladonna alkaloids. This treatment, in fact, was the basis of the pharmacological approach to the disease for many years. The efficacy of the anticholinergic drugs and the detrimental effect of cholinomimetics has been interpreted as evidence for an acetylcholine-DA inbalance in Parkinson's disease (Barbeau, 1962). Anticholinergics, however, are also able to block uptake of DA into nerve endings (Coyle and Snyder, 1969a), and might conceivably be operating solely on dopaminergic neurones to produce their antiparkinsonian effect.

Amphetamine has also been used with some success to treat parkinsonism, and is generally thought to act indirectly

on dopaminergic neurones (see Chapter 6).

Although its mechanism of action is far from clear, amantadine has been found to help certain patients with Parkinson's disease (Schwab et al, 1969).

DA receptor agonists are another class of drug which have been used to treat Parkinson's disease, and when they were introduced, hopes were high, for their effects do not depend on metabolism, or on an intact nigro-striatal pathway. All that is required are functional DA postsynaptic receptors on which the drug can impinge. However, these compounds did not live up to their original promise, although some patients have derived benefit from them. Bromocriptine is the most widely used of these drugs but, in general, its antiparkinsonian potency does not surpass that of L-dopa. In fact, both drugs have been used together with some success (Pearce and Pearce, 1978). DA receptors have recently been classified into two populations (Kebabian and Calne, 1979), but it remains to be seen whether increasing the specificity of DA agonists will produce drugs of any further benefit to the parkinsonian patient.

(c) MAO inhibitors

Several studies have shown that the increase in animal brain catecholamine content following L-dopa could be augmented by the co-administration of MAO inhibitors (e.g. Carlsson et al, 1958; Weil-Malherbe et al, 1961). MAO inhibitors alone, in fact, have been reported to increase the concentration of DA in the brain (Holzer and Hornykiewicz, 1959). If MAO inhibitors had the same effects in the parkinsonian brain, they would constitute an alternative treatment of the disease, or administration of a combination of L-dopa with a MAO inhibitor may result in extra benefit to the patient. Such reasoning led several groups to test the effect of MAO inhibitors alone and with L-dopa (Birkmayer and Hornykiewicz, 1962; Barbeau et al, 1962; McGeer et al, 1961). Whilst the value of MAO inhibitor therapy without L-dopa was modest or even without value (Chesrow et al, 1962; Rosen, 1969), it was generally agreed

that the antiparkinsonian effect of L-dopa was potentiated when the two drugs were given together.

The MAO inhibitor, harmine, had in fact been used many years previously in the treatment of Parkinson's disease (Behringer and Wilmanns, 1929) although it is possible that the benefit did not derive from inhibition of MAO, as a related compound harmalol which does not affect MAO was also claimed to be beneficial (Cooper and Gunn, 1931).

A study on the brains of patients who died whilst taking MAO inhibitors only revealed that DA concentrations were only very slightly raised above those found in untreated parkinsonians (Bernheimer et al, 1963). However, the authors found that 5HT and NA concentrations were substantially elevated possibly because, unlike the dopaminergic neurones, the serotonergic and noradrenergic neurones were intact.

However, the use of MAO inhibitors to treat the disease was discontinued when certain side effects came to light (Birkmayer, 1966), including hallucinations, anxiety, delusions and confusion. McGeer et al (1961), Barbeau et al (1962) and Hunter et al (1970) were among those who reported that large rises in blood pressure occurred in patients receiving an MAO inhibitor in addition to L-dopa therapy. This adverse reaction is essentailly a variant of the "cheese effect" (Chapter 3).

When the selective MAO inhibitor, deprenyl, became available to the clinician, Birkmayer et al (1975) studied its antiparkinsonian properties alone or with L-dopa therapy in an unblind fashion. Very encouraging results were reported by this group. Deprenyl was found to potentiate the anti-akinetic effect of L-dopa, and markedly to reduce the incidence of "on-off" reactions, the term given to a variety of distressing fluctuations in ability to initiate movement from which many parkinsonians treated for long periods of time with L-dopa suffer. Deprenyl was reported to be quickly acting when given with L-dopa and a decarboxylase inhibitor, but to be inactive when administered

without a decarboxylase inhibitor, although the apparent failure of the drug in these instances has not been referred to since by the group (Birkmayer et al, 1977: Birkmayer, 1978). The value of deprenyl alone was not clear from their results. However, an important observation was the absence of hypertensive reactions in any patients in the trial. This feature of deprenyl has since been rigorously tested (Chapter 3).

A double blind trial was urgently needed in order to substantiate the report of Birkmayer et al (1975). Therefore, in conjunction with Dr. Gerald Stern's team at University College Hospital, a careful study was made of the effects of oral deprenyl on different clinical manifestations of the "on-off" phenomenon and its antiparkinsonian properties alone or with L-dopa therapy. In view of the possible antidepressant action of deprenyl, a self-rating assessment was used to examine mood changes and emotional state. Biochemical measurements were also made. Platelet MAO values were monitored in a number of cases to gauge the speed and extent of the inhibitory effect of deprenyl, and also to see whether there was any clinical correlation with these parameters. The consequence of deprenyl administration on PEA oxidation was investigated by measuring the urinary excretion of the amine and its metabolite PAA.

Methods

(i) Clinical

Forty-six patients were included in the double blind crossover trial; 41 received maximum tolerated doses of L-dopa alone or with a decarboxylase inhibitor, and 5 were previously untreated.

Those with "on-off" effects rated their own daily disability using self-scoring diaries. These assessments were checked during a day when the patient remained on the ward. All patients were seen every two weeks as outpatients.

(ii) Biochemical

Platelet MAO activities were measured as described in Chapter 2. The blood samples were collected at the weekly clinic, and the platelets were either harvested at the hospital, or brought back to the laboratory for processing.

The methods used for determining the urinary content of PEA and PAA are described in Chapter 2. Reliable 24 h urine collections are difficult to obtain from old people in general and from parkinsonian subjects in particular on an outpatient basis. Therefore, even though it meant limiting the number of samples, only those collected under nursing supervision from hospital patients were collected.

A number of routine liver function tests was carried out on patients receiving deprenyl.

Results

(i) Clinical

The patients whose disabilities were fairly constant throughout the day derived no significant benefit from the addition of deprenyl to their existing L-dopa therapy.

In the other group of patients who did experience an "on-off" type of disability, there was no overall improvement in mean daily akinesia or dyskinesia scores. However, when the various forms of "on-off" disability were individually accounted, some improvements due to deprenyl became apparent (Table 4:1). No difference in response to deprenyl was detected between the subjects taking L-dopa alone and those receiving L-dopa with a decarboxylase inhibitor. The addition of deprenyl to L-dopa therapy allowed a reduction in the dose of L-dopa without concomitant deterioration in benefit. In doses up to 15 mg daily, deprenyl had no antiparkinsonian properties in the patients receiving no other drug treatment, but rapid benefit took place after the introduction of L-dopa therapy. In those subjects who responded well to deprenyl, treatment was found to be just as effective when given on alternate days only.

Table 4:1

The response of on-off disturbances to deprenyl

Akinetic Disabilities			Dyskinetic Disabilities		
Disability	No. Affected	No. Improved	Disability	No. Affected	No. Improved
Rapid oscil-lations (yo-yo effects)	8	2	Peak dose	22	0
			End-of-dose dyskinesia	1	0
End-of-dose akinesia	19	12	Early-morning dyskinesia	2	0
Freezing episodes	14	0			
Early-morning akinesia	16	9			
Nocturnal akinesia	13	9			

No statistically significant antidepressant effect was found, but it is worth noting that three patients with mild agitated depression did experience a sustained antidepressant effect whilst taking deprenyl and suffered a relapse when receiving placebo. The onset of improvement after deprenyl usually occurred within 72 h of starting treatment with the drug.

Dyskinesias were the most common side effect (Table 4:2), though this, like all the other side effects, could be reversed by reducing the dose of deprenyl.

No evidence of hepatotoxicity was found in any patient taking deprenyl for up to six months.

Even though more patients at University College Hospital have received deprenyl since the completion of this trial, the overall picture has not changed (A.J. Lees personal communication).

(ii) Biochemical

The results from the platelet MAO activity determinations are shown in Table 4:3, together with a clinical assessment of the benefit derived. Not all subjects proved equally susceptible to the inhibitory action of deprenyl, but several days treatment at a dose as low as 3 mg a day reduces platelet MAO activity to 10% of baseline level. Those patients who seemed less sensitive to MAO inhibition did not necessarily show a poorer clinical response (but see below).

Samples obtained during placebo phases indicated that recovery of enzyme activity after total inhibition takes 2 to 3 weeks.

The effect of a single 10 mg dose of deprenyl was studied in 3 fasting patients (Figure 4:1). These results reveal that deprenyl has a very rapid inhibitory effect on the platelet enzyme. An equilibrium is reached after 2 to 3 h, and then remains unaltered for as long as 48 h. The subject whose platelet MAO activity was least affected by deprenyl also derived least benefit from the drug. However, this could easily have been a chance occurrence.

Table 4:2

Frequency of side-effects

Side effect	No. of patients
Dyskinesias	14
Nausea	9
Dry mouth	6
Dizziness	3
Postural hypotension	2
Syncope	1
Circumoral paraesthesia	1
Hallucinations	1
Unpleasant taste	1

Table 4:3

Effect of deprenyl on platelet MAO activity and parkinsonism

Patient	Treatment (Daily Deprenyl Dose or Placebo)	Length of Treatment (Chronological order)	Inhibition of Platelet MAO (% of baseline)	Eventual Clinical Response to Deprenyl	Type of Disability Helped
1	3 mg	1w	100		
	Placebo	3w	0		
	10 mg	2w	100	Moderate	Park.
	10 mg	4w	100		
	10 mg	7w	100		
2	10 mg	1w	100	Good	Park.
	Placebo	2w	10		
3	3 mg	1w	94	Poor	
	Placebo	1w	58		
4	3 mg	1w	96	Good	ED
	10 mg	6w	99		
	Placebo	2w	42		
5	5 mg	4w	100	Good	ED
	Placebo	2w	0		
6	10 mg	2w	100	Good	ED
	Placebo	2w	48		
7	10 mg	2w	99	Moderate	ED

(continued)

Table 4:3 (cont.)

Patient	Treatment (Daily Deprenyl Dose or Placebo	Length of Treatment (Chronological order)	Inhibition of Platelet MAO (% of baseline)	Eventual Clinical Response to Deprenyl	Type of Disability Helped
8	10 mg	6w	100	Moderate	ED
9	10 mg	6w	100	Poor	
10	10 mg	2w	99		
	10 mg	6w	100	Poor	
	Placebo	2w	85		
11	10 mg	2w	100	Moderate	ED
12	10 mg	2w	100	Poor	
13	10 mg	2w	100	Good	ED
14	5 mg	2d	4	Poor	
15	5 mg	1h	56	Good	ED
16	5 mg	2d	100		
	5 mg	6d	100	Poor	
	10 mg	1w	100		

Platelet MAO activity in each sample was measured at least twice, each time in duplicate and the values shown represent the mean of the determinations.

Abbreviations: week (w), day (d), hour (h), end-of-dose deterioration (ED), general parkinsonism (Park.).

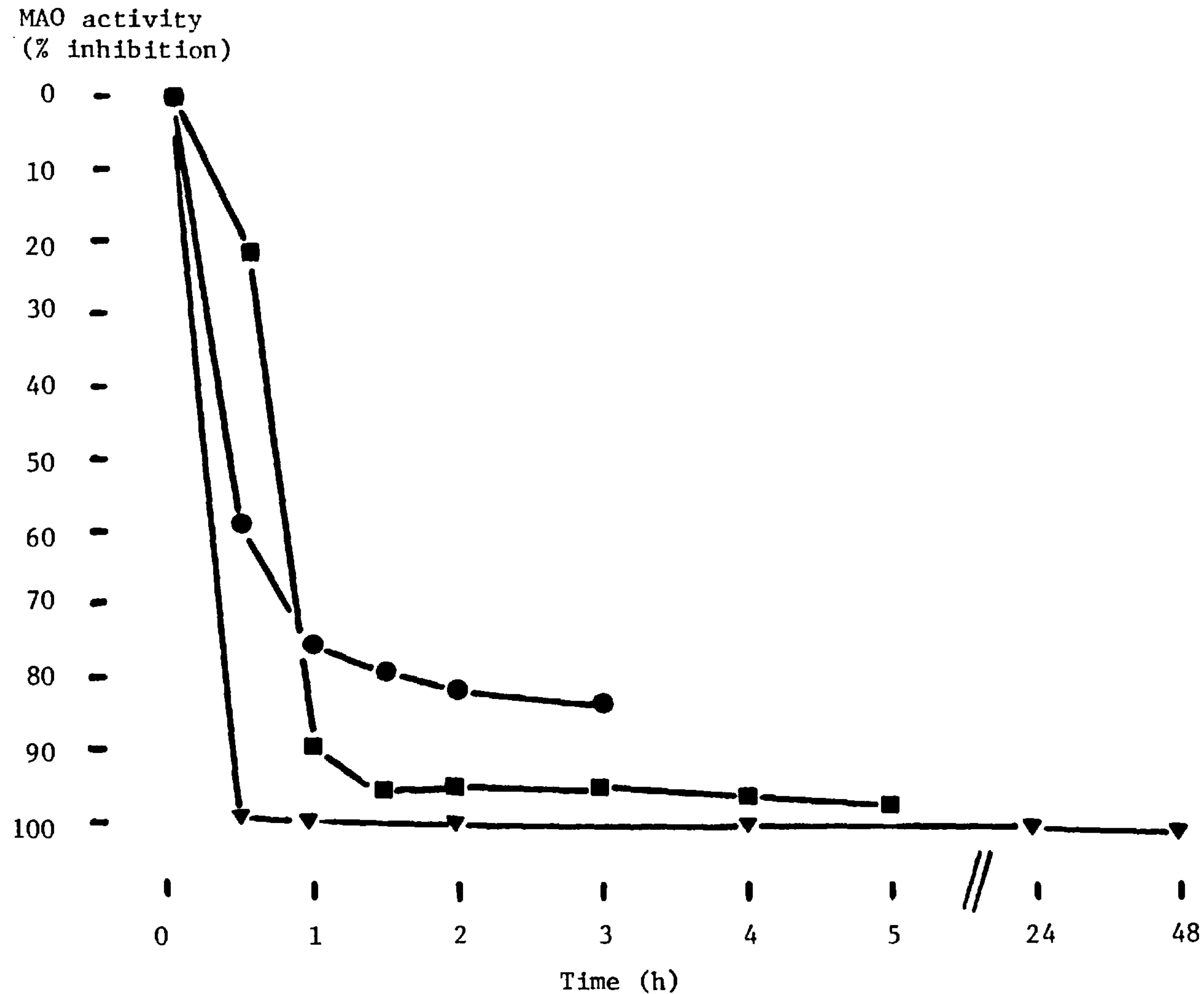


Fig. 4:1 Time course of inhibition of platelet MAO by oral deprenyl.

Results from 3 previously untreated parkinsonians; 0 h is the time patients were administered 10 mg deprenyl.

Untreated parkinsonian patients were found to excrete a mean (±SEM) of 4.40±1.50 μg per 24 h of free PEA, which is not significantly different from the value determined for normal subjects (Chapter 3) of 4.99±0.44 per 24 h. Those patients receiving L-dopa with carbidopa had a mean urinary PEA concentration of 2.55±0.87 μg per 24 h which was not significantly different from those subjects receiving deprenyl in addition (3.49±0.73 μg per 24 h), but was significantly lower than the normal value (4.99±0.44 μg per 24 h) (Table 4:4). The patients taking deprenyl alone or just L-dopa (without carbidopa) and deprenyl had markedly elevated levels of urinary PEA (Table 4:5).

The presence of a deprenyl metabolite peak (amphetamine, Chapter 6) in the chromatogram containing the PEA peak, was taken as confirmation that patients were receiving deprenyl.

The serial samples collected from 5 parkinsonians showed that the absence of an alteration in PEA excretion in patients taking deprenyl in addition to dopa and carbidopa was due to the presence of the decarboxylase inhibitor (Table 4:5). Patient A, who was previously untreated, excreted a greatly increased amount of PEA when he started to take deprenyl alone. Patient B, who had also received no antiparkinsonian drugs, produced a raised urinary PEA concentration on a dose of 10 mg deprenyl daily, which rose further as the dose of deprenyl was stepped up to 15 mg a day. Patient C was receiving just L-dopa initially, and his PEA output was markedly elevated after 3 days treatment with 10 mg deprenyl daily, and it rose still further after 3 days at 20 mg a day. His dose then reverted to 10 mg a day and after 6 months, another sample was collected, which had an even higher content of PEA. Another previously untreated patient, D, had a urinary PEA concentration which rose as the dose and length of deprenyl treatment was increased. However, when the patient's medication was altered, to include L-dopa and a peripheral decarboxylase inhibitor, the urinary PEA concentration fell immediately. Patient E was receiving L-dopa with a decarboxylase inhibitor from the outset.

Table 4:4

Free urinary PEA excretion in Parkinson's disease

Patient	L-Dopa	Carbidopa	Deprenyl	PEA (µg/24h)
n=8	-	-	-	mean ±SEM 4.40±1.5
n=4	√	√	-	mean ±SEM 2.55±0.8
1	√	√	2y 7.5mg	4.40
2	√	√	5w 10mg	7.58
3	√	√	2d 10mg	1.36
4	√	√	11w 10mg	3.68
5	√	√	4w 5mg	2.06
6	√	√	16w 10mg	4.05
7	√	√	1w 10mg	1.14
8	√	√	1w 10mg	1.30
9	√	√	2y 5mg	2.37
10	√	√	2y 15mg	6.96
				mean ±SEM 3.49±0.73

Each value represents the mean of at least two determinations.

Abbreviations: year (y), week (w), day (d).
√ patient receiving drug
- patient not receiving drug

Table 4:5

Effect of carbidopa on free PEA excretion in Parkinson's disease

Patient	Dopa	Carbidopa	Deprenyl	PEA (μg/24 h)
A	-	-	-	0.48
	-	-	1d 10 mg	57.38
B	-	-	-	0.49
	-	-	1d 10 mg	0.90
	-	-	3d 10 mg	27.46
	-	-	4d 10 mg	22.02
	-	-	1d 15 mg	62.08
	-	-	2w 15 mg	81.90
C	√	-	-	4.95
	√	-	3d 10 mg	89.71
	√	-	3d 20 mg	270.52
	√	-	6m 10 mg	407.10
D	-	-	-	1.74
	-	-	1d 10 mg	6.11
	-	-	1d 30 mg	19.15
	-	-	1d 60 mg	67.29
	-	-	3d 60 mg	108.96
	√	√	1d 60 mg	20.80
E	√	√	-	4.82
	√	√	1d 10 mg	3.36
	√	√	1d 20 mg	5.07
	√	√	1d 30 mg	16.35
	√	√	2y 45 mg	16.97

Each value represents the mean of at least two separate assays.

Abbreviations: day (d), week (w), month (m), year (y).
√ patient receiving drug
- patient not receiving drug

The addition of deprenyl at doses of 10 and 20 mg daily did not appreciably change the PEA concentration, although doses of 30 mg and 45 mg daily for prolonged periods did raise this value.

There was no significant change in free PAA excretion following deprenyl administration (Table 4:6).

Discussion

The clinical results from the trial indicated that deprenyl was only of value to those patients experiencing certain "on-off" disabilities, end-of-dose akinesia, early morning akinesia and nocturnal akinesia (Table 4:1). The outcome of the trial was not as encouraging as the reports of Birkmayer et al (1975, 1977) and Birkmayer (1978). This group found that deprenyl potentiated the antiparkinsonian properties of L-dopa therapy, apart from preventing the occurrence of the "on-off" phenomena in the early stage of the disease, and reducing the side effects of conventional treatment by allowing a lower dose of L-dopa to be used.

Birkmayer's group has employed submaximal doses of L-dopa with benserazide in the treatment of Parkinson's disease from the outset in the belief that many of the long term side effects of L-dopa might then be avoided. In fact, their incidence of "on-off" effects after long-term L-dopa therapy was claimed to be as low as 2.9% (Birkmayer, 1976), unlike the values reported by other groups (Sweet and McDowell, 1975).

The very favourable results achieved with deprenyl by the Austrian workers may well stem from their policy of administering submaximal doses of L-dopa. The addition of the MAO inhibitor may, in effect, be producing just the extra benefit which their patients would derive from an increase in L-dopa dosage, albeit by a different mechanism. Patients receiving the maximum tolerated dose of L-dopa, as in the present trial, would have no capacity for a further increase in benefit.

Rinne et al (1978) in an unblind trial did report additional benefit in most patients given deprenyl in

Table 4:6

Effect of deprenyl on free PAA excretion in Parkinsonians

Patient	Free PAA Excretion (mg/24h)					
	When Receiving			With Addition of Deprenyl		
	Dopa	Carbidopa		(length)	(daily dose)	
1	√	-	0.48	1y	10mg	0.50
2	√	√	0.32	2w	10mg	0.69
3	√	√	1.02	2y	5mg	0.39
4	√	√	1.10	2w	45mg	3.10
5	√	√	0.17	1w	10mg	0.79
6	√	√	0.90	1w	10mg	0.83
7	√	√	0.50	4w	10mg	1.16
8	√	√	0.31	2w	15mg	0.19
			$\overline{X}$=0.60			$\overline{X}$=0.95

Each value represents the mean of duplicate determinations.

Abbreviations: week (w), year (y).
√ patient receiving drug
- patient not receiving drug

combination with an "optimal" dose of L-dopa with or without a decarboxylase inhibitor; all akinetic but not dyskinetic, disabilities contributing to the "on-off" effects were helped in addition. In the present study dyskinetic disabilities, similarly, were unaffected but some of the akinetic components of the "on-off" phenomena were also unimproved, freezing episodes and "yo-yo" oscillations. In other trials of deprenyl in Parkinson's disease (Csanda et al, 1978; Yahr, 1978) no detailed distinction was made between the various types of "on-off" effects which no doubt result from different predisposing factors (Marsden and Parkes, 1976).

Of all the investigations so far reported, only Csanda et al (1978) have claimed to find any antiparkinsonian action of deprenyl when used alone; however, those patients who did benefit were afflicted with mild parkinsonism only. Inhibition of MAO prolongs and potentiates the synaptic effects of amine transmitters, so that the administration of deprenyl alone might perhaps be of help in mild cases where more intact nigro-striatal neurones exist than in advanced cases. The combination of deprenyl with L-dopa treatment in the present study tended to prevent end-of-dose deterioration and nocturnal and early morning akinesia, complaints which are all likely to result from DA insufficiency in the striatum. Marsden and Parkes (1976), in fact, predicted that these types of "on-off" effects would respond to a long-acting L-dopa preparation, which is what deprenyl with L-dopa essentially is.

The potent effect which oral deprenyl had on platelet MAO activity in parkinsonian patients (Table 4:3; Figure 4:1) was similar to that observed in normal subjects (Chapter 3). These results are also in agreement with those of Birkmayer et al (1975, 1977) and Riederer et al (1978). This group reported that one intravenous or oral 10 mg dose of deprenyl inhibited platelet MAO activity in parkinsonian patients by more than 90% within 30 or 90 min, respectively. This level of inhibition was found to be maintained for up to 24 h. They also claimed to observe benefit in parkinsonian patients coinciding with maximum inhibition of the platelet

enzyme following a dose of deprenyl. If this were to be confirmed on a blind basis, it would be good evidence that platelet MAO mirrors central enzyme activity. However in the clinical trial reported here, even though patients differed in their susceptibility to platelet MAO inhibition, there was no correlation of this effect with clinical findings. Nor could the time at which maximum platelet inhibition was attained, 2 to 3 h (Figure 4:1), be linked with the onset of benefit. Patients who improved on deprenyl did so, usually, within 72 h. Yahr (1978) also reported that a response to deprenyl was evident within 3 days of adding the drug to L-dopa therapy.

Some patients in the present study only derived benefit from deprenyl when the dose was raised from 5 to 10 mg a day, whereas increasing the dose above 10 mg daily produced no extra improvement. On explanation of this may be that at the lower dose, a large proportion of the drug is metabolized (Chapter 6) before reaching important sites, whereas at higher dosage more is free to combine with MAO.

Patients who responded well to daily treatment also found that alternate day treatment with deprenyl was satisfactory. This was not unexpected, as platelet MAO activity remains undetectable the day after complete inhibition is achieved (Figure 4:1). However, this regimen was not generally used because lower patient compliance was anticipated.

The normal PEA excretion in patients taking deprenyl along with carbidopa and dopa, and the reduced output in those taking just carbidopa and dopa, is evidence that amino acid decarboxylase is the enzyme responsible for the conversion of phenylalanine to PEA in man. In vitro studies have shown that PEA is formed from phenylalanine by this enzyme (Lovenberg et al, 1962). Furthermore, an inhibitor of the enzyme has been shown to prevent the conversion (Silkaitis and Mosnaim, 1976). In an experiment parallel to the situation in our parkinsonian patients, Dyck and Boulton (1975) found that a MAO inhibitor raises rat urine PEA concentration, but adding a decarboxylase inhibitor caused a reversion of PEA output to normal.

Even so, Table 4:5 shows that parkinsonian patient E did have a slightly raised output of PEA on high deprenyl dosage in combination with L-dopa and a decarboxylase inhibitor. This observation, together with the further increase in urinary PEA concentration of subjects C and D following large amounts of deprenyl, suggest either that traces of MAO-B activity remain when the lower doses of deprenyl are employed even when platelet activity is undetectable *in vitro* or that PEA is to some extent deaminated by MAO-A *in vivo* and this enzyme form is inhibited by large concentrations of deprenyl. Such interpretations must obviously be tentative, bearing in mind the small sample number involved.

The absence of elevated PEA excretion in patients being treated with deprenyl and carbidopa (Table 4·4), a decarboxylase inhibitor which does not cross the blood-brain barrier, indicates that urinary PEA reflects peripheral, not central, production of the amine. This conclusion received support from Reynolds et al (1978), who found an increased PEA concentration in the post-mortem brains of parkinsonians who had been under treatment with deprenyl in conjunction with L-dopa and a peripheral decarboxylase inhibitor.

Much research has recently been directed towards PEA, an amine normally present in brain and possessing biological activity in its own right. Furthermore various authors have suggested its involvement in the aetiology of affective disorders such as depression (e.g. Sabelli et al, 1978a). Although it may possess a direct agonist action (Antelman et al, 1977), PEA is generally thought to act indirectly. It is known to induce behavioural changes similar to those of amphetamine (Mantegazza and Riva, 1963), to which it bears a close structural resemblance. The stereotyped behaviour induced by PEA is blocked by α-methyl p-tyrosine (Braestrup et al, 1975) suggesting, perhaps, that the effect is dependent on catecholamines. PEA releases DA *in vivo* and *in vitro* more effectively than NA (Baker et al, 1976), and is also a more potent inhibitor of the DA uptake, compared with that of

NA (Horn, 1973). Physiologically, it has been proposed that PEA functions as a neuromodulator and/or a co-transmitter (Sabelli and Mosnaim, 1974) and also that under certain circumstances it could be a precursor to DA (Silkaitis and Mosnaim, 1976). PEA has an extremely low Km value towards MAO-B, about 6 μM (Chapter 7), so that raised concentrations of the amine might by substrate competition, retard the degradation of other MAO substrates, such as DA, thereby enhancing the action of the protected substance. It is evident, therefore, that the elevated concentration of PEA which develops following deprenyl treatment, could contribute to the effects of the drug. It is especially interesting to note here that PEA has been found to be concentrated in the striatum (Jackson, 1978) and that D-phenlyalanine has been reported to relieve some of the symptoms of Parkinson's disease (Heller, 1978). A decrease in urinary PEA in this condition has been put forward as evidence for the involvement the amine in extrapyramidal function (Heller and Fischer, 1973; Sabelli et al, 1978b); however, the methods used were relatively non-specific (Schweitzer et al, 1975) Furthermore, Heller and Fischer (1973) just withdrew anti-parkinsonian drugs from their patients for 12 days prior to urine collection, Sabelli et al (1978b) do no give any patient details whatsoever. Therefore, these studies do not provide reliable information on the urinary excretion of PEA in untreated parkinsonians.

The absence of any significant change in the urinary concentration of PAA in Parkinson's disease following deprenyl treatment (Table 4:6). probably stems either from the conjoint effect of a decarboxylase inhibitor in the subjects studied, or from the masking of any alteration by the large amounts of PAA normally present in urine (Chapter 3).

In Knoll's (1978) view, the beneficial effect of deprenyl in Parkinson's disease results from its activation of nigro-striatal dopaminergic neurones which have been "subdued" as a result of stimulation of DA autoreceptors by the large amounts of amine formed from L-dopa. His pharmacological studies, albeit utilising big doses of deprenyl, indicate that this activation is achieved by blockade of DA uptake as well as by inhibition of intraneuronal MAO-B.

In the unilateral 6-hydroxydopamine nigrotomized rat model, deprenyl produces a mild ipsilateral rotation reminiscent of amphetamine when given alone, but enhances the contralateral rotation produced by L-dopa (Yahr, 1978). Methamphetamine and amphetamine are metabolites of deprenyl (Chapter 6), and it has long been known that amphetamine can relieve parkinsonism (Solomon et al, 1937). Although it is probably the laevorotatory isomer which is generated from laevo-deprenyl (Chapter 6), even laevo-amphetamine has been found to produce significant improvement in parkinsonian disability (Parkes et al, 1975).

Thus, even though the antiparkinsonian effects of deprenyl seem likely to derive largely from inhibition of DA oxidation, other factors, such as its amphetamine metabolites and raised PEA concentrations also deserve consideration.

CHAPTER FIVE - THE EFFECT OF DEPRENYL ON DA OXIDATION IN HUMAN BRAIN AND PERIPHERAL TISSUES

(i) MAO in brain

The linking of monoamine systems with such disorders as depression (Schildkraut, 1965), Parkinson's disease (see Chapter 4) and schizophrenia (Snyder, 1972) inevitably stimulated research into the metabolism of compounds such as NA, 5HT and DA. Much of this interest has been directed towards MAO. Activity of this enzyme has been measured in various regions of post-mortem brains in the hope that a difference between patients with various conditions and normal subjects would be apparent (Utena et al, 1968; Vogel et al, 1969; Grote et al, 1974; Schwartz et al, 1974a). However, no convincing defect emerged. All of these studies employed just one substrate, but as interest in the multiple forms of MAO grew, more experiments using two or more substrates were carried out in order to examine the relative distribution of MAO-A and MAO-B. Most of these investigations were performed on animals, for example the rat (Suzuki and Yagi, 1976), hamster (Edwards and Malsbury, 1977) and vervet monkey (Murphy et al, 1979). Whilst such studies are interesting, because of the limited amount work it has been possible to carry out on human brain, there is very little evidence to support any claims that these animals are suitable models for man. Indeed, the work performed on human brain MAO indicates that a very significant difference exists between DA oxidation in rat and man. In the rat, Yang and Neff (1974) found that DA is deaminated *in vitro* by both forms of MAO. However, Braestrup et al (1975) and Waldmeier et al (1976) showed that *in vivo* DA is metabolized primarily by the A form. Green et al (1977), who also worked in this species, concluded that DA is predominantly oxidised by MAO-A, but that both forms of the enzyme need to be extensively inactivated before L-dopa produces a rise in brain DA concentration or induces hyperactivity. However, *in vitro* studies of Glover et al (1977) on human brain showed that DA is predominantly metabolized by MAO-B. Although the findings of White and Glassman (1977) and Roth and Feor (1978), who found DA to be a substrate for both forms of MAO in human brain, appear at first to be contradictory to the report of Glover et al (1977), the results from these two groups indicate that DA is in fact mainly oxidised by the B form. Furthermore, they limited their estimations to the cortex.

(ii) Mixed substrate experiments

All the studies so far cited in this chapter used either the preferential oxidation of substrates or the selective action of inhibitors to distinguish between the forms of MAO. Several workers, however, have used the method of mixed substrates (Dixon and Webb, 1958) to investigate the different types of MAO in human brain. This involves incubating pairs of substrates with the enzyme and observing the type of inhibition one exerts on the other. The data obtained then indicates whether they share the same catalytic site on the enzyme. White and Wu (1975) used this technique on a preparation of human cortex and found that whilst PEA and 5HT are metabolized at independent sites as anticipated, the oxidation of the other substrates they tested cannot be explained satisfactorily on the basis of these two sites only. They postulated the existence of a third site at which NA and DA are partly metabolized, although DA is also oxidised at the same site as PEA to some extent. Another mixed substrate study (Edwards and Chang, 1975) indicated that the platelet enzyme is not homogenous, but comprises several isoenzymes or contains multiple catalytic sites. This interpretation was based on the non-competitive mode of inhibition exerted by PEA on the oxidation of tryptamine or BZ, whilst tryptamine and BZ are competitive inhibitors of each other. Furthermore, tryptamine or BZ are non-competitive inhibitors of PEA metabolism. Roth (1976) obtained similar results to those of Edwards and Chang (1975) using human brain and the method of mixed substrates, but found BZ and PEA to be identically inhibited by two selective MAO inhibitors. Roth (1976) resolved this paradox by discovering that BZ and PEA become competitive inhibitors of each other when the assay is conducted at an elevated oxygen concentration. Such a result is consistent with PEA and BZ sharing a common site on MAO, but in addition binding to an inhibitory site on the reduced form of the oxidase. At high concentrations, oxygen, it was proposed, saturates the reduced form and prevents amines from binding. This study, therefore, sheds some light on the otherwise confusing data of White and Wu (1975) and Edwards and Chang (1975). It is appropriate to mention here that Houslay et al (1974) reasoned that mixed substrate experiments do not necessarily indicate the presence of more than one species of enzyme if the Km values of two enzymes are similar

for an individual substrate.

Recently several authors (Fowler et al, 1978; Murphy, 1978; Lewinsohn et al, 1980) have recommended that when classifying MAO into A- and B-forms, several substrates should be used together with a selective inhibitor. In the present study, besides examining the relative rates of deamination of 5HT, NA and DA, the effect of the selective MAO inhibitor, deprenyl, on DA metabolism was investigated in various regions of human brain and also in several peripheral tissues. In view of the value of deprenyl as an adjuvant to the L-dopa therapy of Parkinson's disease (see Chapter 4), particular attention was paid in this study to the effect that deprenyl has on *in vitro* DA oxidation in the striatal regions of the brain in an effort to clarify the drug's mode of action.

Materials

Domestic meat slicer - Robert Krups Ltd., London, U.K.

Brain mould - gift from Division of Psychiatry, Clinical Research Centre, Harrow, U.K.

Methods

(i) Collection of material

All tissues used were of human origin.

Jejunal tissue was obtained at biopsy. Samples were taken for diagnostic purposes and those used in this study found to be normal. After removal from the body, they were immediately placed on solid carbon dioxide ($-80^{o}C$).

Placentae were collected within 1-6h of delivery (by Dr. Dr. R. Lewinsohn), and then rinsed in normal saline before freezing in solid carbon dioxide.

All other tissues, with the exception of platelets, were obtained at autopsy from subjects without disease of the nervous system. Samples from 5 individuals (4 male, 1 female) were collected between 24 and 48 h after death up till which time the bodies had been stored at $4^{o}C$. A sample of each peripheral tissue to be examined was taken as soon as it had been removed from the body, and after washing in normal saline was frozen on solid carbon dioxide. Brains were treated similarly except that before freezing they were placed in a polystyrene mould on removal from the cranium.

This preserved their shape, thus aiding eventual dissection.

All the material described above remained in solid carbon dioxide until transferral to permanent storage at -20°C.

Collection and harvesting of platelets was described in Chapter 2.

(ii) Dissection of brains

This technique was acquired during several visits to the Division of Psychiatry, Clinical Research Centre, Harrow. The method has subsequently been published (Owen et al, 1979).

The brain was removed from storage and allowed to warm to about -10°C prior to dissection. Before sectioning the brain, samples from the required cortical areas were obtained by shaving off tissue at the appropriate places. Coronal slices were made using a domestic meat slicer. The width of each cut varied from about 1 cm initially down to about 0.2 cm upon reaching regions from which material was to be taken for further examination. The slices were kept on glass tiles which lay on a bed of ice. The required areas were then dissected out, guided by the atlas of DeArmond et al (1976). The nucleus accumbens was the most difficult area to dissect with confidence but Figure 5:1 shows the part which was taken. Portions of tissues were diced and thoroughly mixed on a glass tile before transferral to plastic containers and freezing in solid carbon dioxide. They were stored at -20°C until homogenization.

(iii) Homogenization was carried out as described in Chapter 2.

(iv) MAO assay The final concentration of the substrates used in the MAO assay were 300 μM for 5HT and DA and 125 μM for PEA.

All chosen regions of a single brain were assayed at the same time to ensure that comparisons of the relative activities were valid. Samples from different individuals were measured on different days.

Two of the 5 brains were analyzed by Dr. V. Glover.

The concentrations of DA used to find the Km values ranged from 60 to 600 μM.

The methods for determining the specific activities and deprenyl sensitivity of MAO were described in Chapter 2.

Protein concentration was measured by the method of Lowry et al (1951) (Chapter 2).

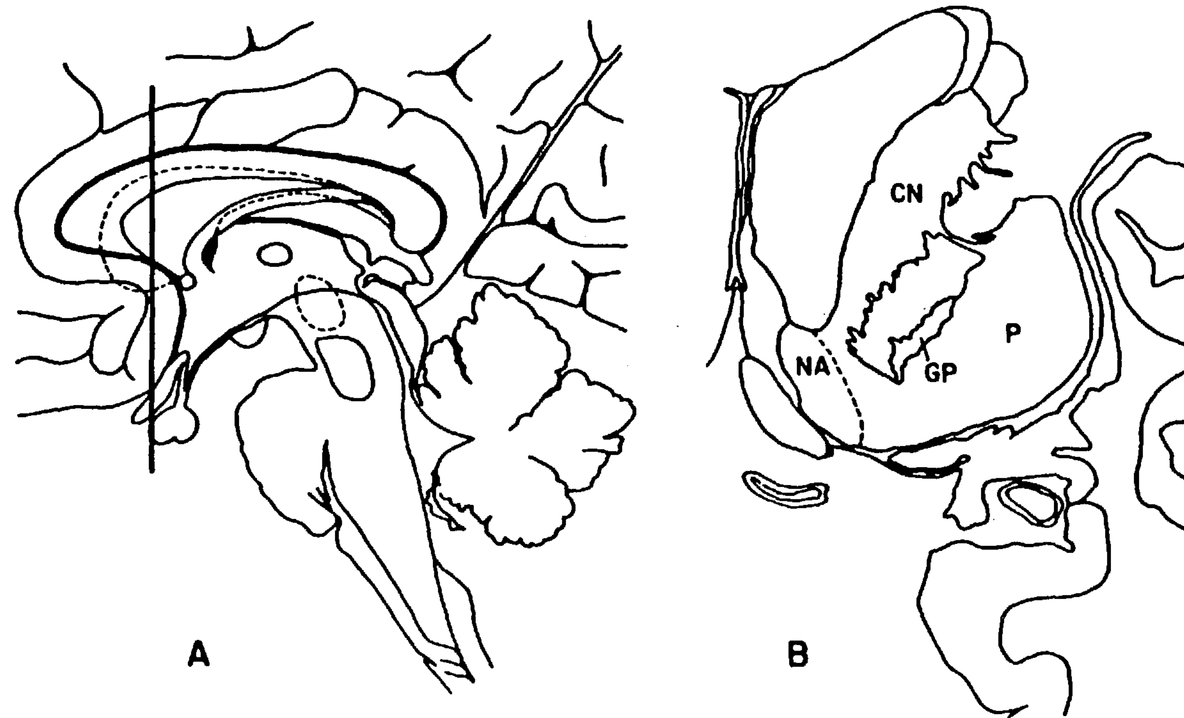


Fig. 5:1 Location of coronal section of brain (A) which reveals (B) the nucleus accumbens (NA), caudate nucleus (CN), putamen (P) and globus pallidus (GP).

Results

Figure 5:2 shows the effect of a range of deprenyl concentrations on the inhibition of MAO-B using PEA as substrate and MAO-A using 5HT in brain homogenates. Samples from different brain regions (temporal cortex and putamen) gave the same inhibition profile. A concentration of 10^{-6} M deprenyl was the most effective at distinguishing between the two forms.

In Table 5:1 the distribution of 5HT, PEA and DA oxidising activities in the various brain areas are shown together with the degree of inhibition of DA oxidation produced by 10^{-6}M deprenyl. The MAO A/B ratio, as reflected by the ratio of the specific activities of 5HT to PEA, varied in the different areas. There was relatively more MAO-B in the DA rich regions of accumbens and striatum than in cortical areas. In areas having a lower MAO A/B ratio, there was also a greater sensitivity of DA oxidation to deprenyl inhibition. The correlation between the PEA/5HT ratio and the percent inhibition caused by deprenyl is 0.84 ($p<0.001$), a result consistent with DA being predominantly metabolized by MAO-B, especially in accumbens and striatum.

The results obtained with the peripheral tissues are shown in Table 5:2. It is apparent that the proportion of MAO-A to MAO-B varies widely throughout the body. The two extremes are the placenta and platelet, which have previously been found to contain solely MAO-A (Egashira, 1976) and MAO-B (Donnelly and Murphy, 1977) respectively. DA oxidising activity is present in both tissues, although relatively higher activity is associated with PEA (DA/PEA) than 5HT (DA/5HT).

The Km values of the enzyme from placenta (130μM) and platelet (140μM) were found to be very similar (Figure 5:3).

Some PEA oxidising activity was observed in the purely MAO-A-containing placenta.

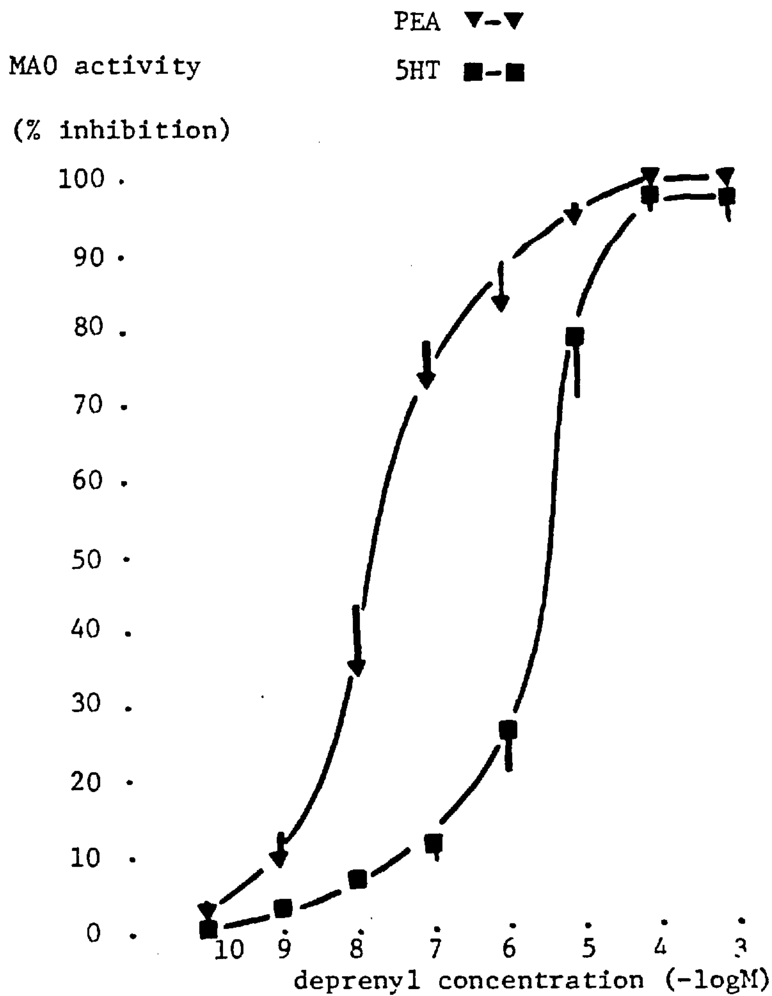


Fig. 5:2 Comparison of deprenyl inhibition of PEA and 5HT oxidation by human caudate

Each point represents the mean (± SEM) of brains from 6 individuals.

Table 5:1

5HT, PEA and DA-oxidising activities in different regions of human brain, and inhibition of DA oxidation by 10^{-6}M deprenyl

Region	5HT	PEA	DA	5HT/PEA	% Inhibition DA oxidation by deprenyl
Accumbens	21.9	21.8	28.5	1.0	83
Caudate	20.2	17.2	29.4	1.2	82
Globus Pallidus	18.0	14.1	21.8	1.3	80
Putamen	16.6	13.1	19.7	1.2	80
Hypothalamus	38.3	22.1	37.2	1.7	70
Thalamus	30.8	16.9	30.7	1.8	72
Amygdala	27.9	14.3	24.8	2.1	73
Precentral cortex	16.7	6.9	13.5	2.4	66
Occipital cortex	20.2	6.0	16.7	3.3	67
Temporal cortex	18.7	8.1	14.5	2.3	65
Frontal cortex	17.6	7.2	12.9	2.4	68
Cerebellar cortex	10.7	4.8	9.6	2.2	63

Results are the means of duplicate assays for 5 brains.

Activities are expressed as nmoles substrate oxidised per mg protein per 30 min.

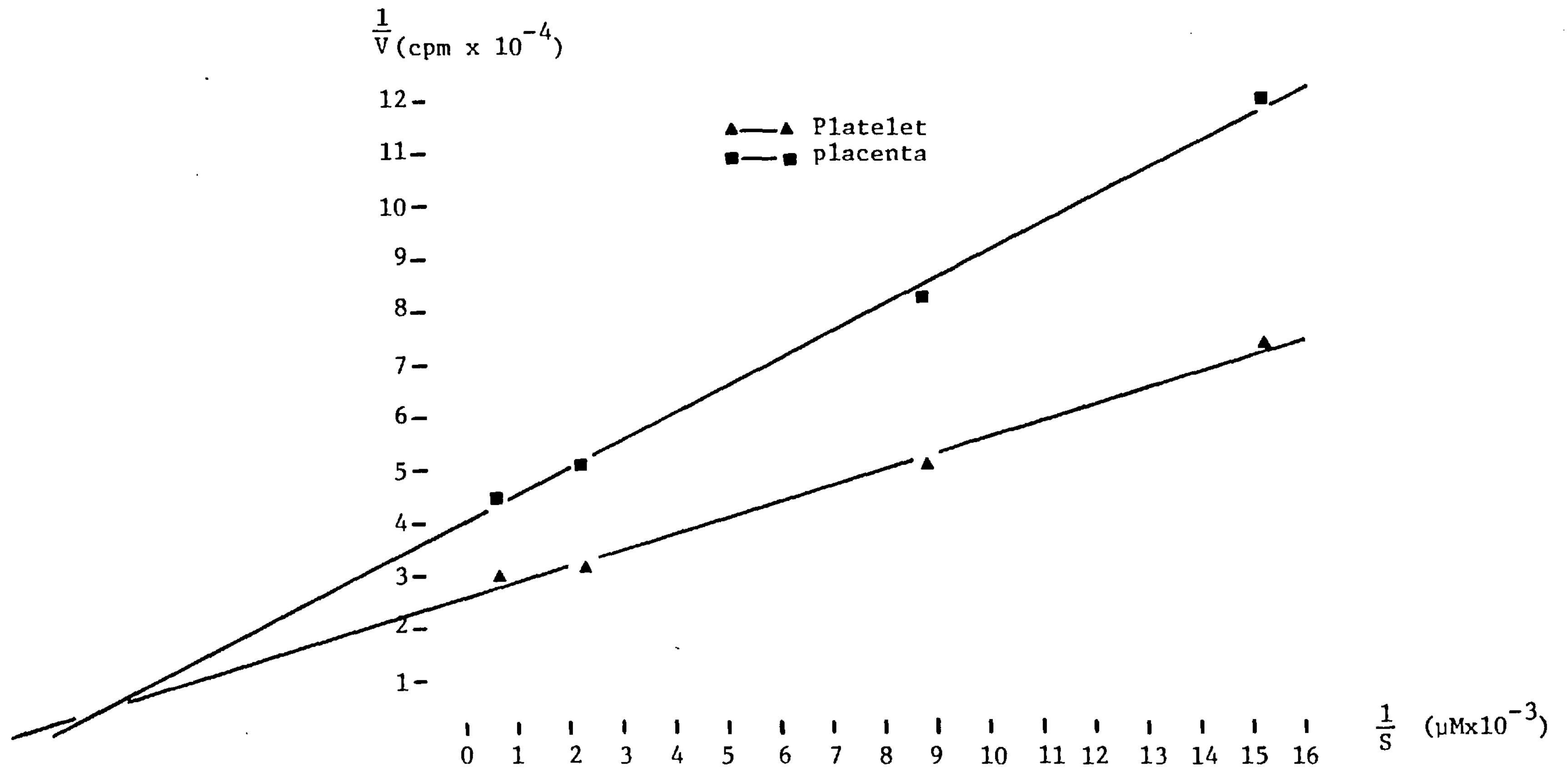
Table 5:2

5HT, PEA and DA oxidising activities in various peripheral tissues and inhibition of DA oxidation by 10^{-6}M deprenyl

Tissue	5HT	PEA	DA	5HT/PEA	% Inhibition DA oxidation by deprenyl
Platelet	0	7.4	5.0	0	98
Heart	4.0	6.1	10.2	0.4	73
Kidney	31.6	27.5	14.5	2.2	64
Liver	52.0	39.7	23.2	2.2	60
Intestine	24.6	11.9	7.6	3.2	35
Placenta	102.4	60.3	29.8	3.4	24
Lung	9.4	9.2	3.8	2.5	22

Results are the means of duplicate assays on 4 individuals.

MAO activity is expressed as nmoles substrate oxidised per mg protein per 30 min.



<u>Fig. 5:3</u> Lineweaver-Burk plots to compare the Km values of placenta and platelet with DA as substrate.

The results represent the mean of 2 assays done in duplicate.

Discussion

The distribution of MAO activity in peripheral tissues agrees well with that found previously (Davison, 1958). As to the different forms of MAO, this study confirms the predominance of the A form in intestine (Squires, 1972; Youdim, 1976) and lung (Lewinsohn et al, 1980). In addition the liver is known to possess slightly more (Youdim, 1976) and the kidney substantially more (Squires, 1972) MAO-B than MAO-A. A novel finding of the present study concerned the heart. Its relative content of MAO-B was second only to the platelet. This result is in contrast to the adult rat heart, which contains mostly MAO-A (Callingham and Lyles, 1975). The significance of the distribution of MAO-A and MAO-B in various organs is unclear. However, it was found that DA, the amine on which this section concentrates, can be metabolized vigorously in all tissues, irrespective of their relative composition of MAO multiple forms. As the Km values for DA with MAO-A or MAO-B (Figure 5:3) are very similar, the proportion of the amine metabolized by the two forms should not vary with substrate concentration. One outstanding attribute of deprenyl is its safety when co-administered with L-dopa, TYR or PEA (Chapter 3). Parkinsonians treated with a MAO inhibitor in addition to L-dopa, with or without a decarboxylase inhibitor, would normally be in danger of suffering a hypertensive episode, as the inactivation of large amounts of peripherally generated DA would be prevented. At least part of the reason why deprenyl can remain safe under these conditions may stem from the ability of several peripheral tissues to oxidise DA via MAO-A. Even though in tissues such as the heart, DA metabolism is very sensitive to deprenyl, the intestine and lung, for example, contain substantial reserves of deprenyl-resistant "DA oxidase."

In the brain the general pattern of activity is consistent with previous studies (Utena et al, 1968; Vogel et al, 1969: Schwartz et al, 1974a; 1974b; Grote et al, 1974; Gottfries et al, 1975; Mackay et al, 1978; Glover et al, 1977; Owen et al, 1979). The most striking similarity between the investigations was that the hypothalamus and the cerebellar cortex nearly always emerged as most and least active areas, respectively. Of the cited studies, only the last two attempted to distinguish between the different forms of MAO. The MAO A/B ratios calculated by these groups (both

on the basis of substrate specificities) agree well with those found in the present study in that a greater MAO A/B ratio was apparent in cortical areas compared with accumbens and striatal areas. The globus pallidus, found here to be similar to the rest of the striatum, was not investigated by Owen et al (1979) or Glover et al (1977). Owen et al (1979) also examined oxidising activity towards DA in the various brain regions, and, based on a significant correlation between this activity and that towards BZ, supported the earlier report (Glover et al, 1977) that DA is a MAO-B substrate in man. They did not, however, combine their tests on substrate preferences with the effect of a selective MAO inhibitor and the present study demonstrates the value of such determinations. Whilst DA is predominantly metabolized by MAO-B in the brain, the A form does have the ability to oxidise DA but to an extent which depends on the actual area. MAO-A contributes more in the cortex than in the striatum, which may explain the apparent discrepancy between the results of White and Glassman (1977) which indicated that DA was a common substrate in the cortex and those of Glover et al (1977) which suggested that MAO-B was almost entirely responsible for its metabolism in the caudate. The pattern of DA oxidation in man is, as mentioned before, unlike that found in the rat. However, in this respect, a similarity does exist between man and the vervet monkey (Murphy et al, 1979) and this animal might, therefore, prove to be a suitable model for human studies. These results on the deprenyl sensitivity of DA oxidation in the striatum provide an explanation for the beneficial effect of the drug in parkinsonism (Chapter 4) as this condition is characterized by DA deficiency in this region.

Attention should be drawn to the not inconsiderable PEA oxidation occurring in the placenta, a tissue which apparently contains only the A form of MAO. In retrospect, it is clear that the concentration of PEA used, 125μM, was rather too large, for at this level the amine is also a substrate for MAO-A (Lewinsohn et al, 1980). It has been suggested in the past that there might be an individual MAO for DA (Youdim, 1972). However, the present results provide no evidence for such a view. This conclusion can be calculated, despite the slight loss of selectivity of PEA in the experiment, if one assumes that the nature of MAO-A and MAO-B are the same in different tissues of an individual. In the purely MAO-A containing tissue, the placenta, DA oxidation proceeds at 0.6 times that of 5HT and in a purely MAO-B containing tissue, the platelet,

DA oxidation proceeds at 1.5 times the rate of PEA (Table 5:2). In all regions of the brain examined, the level of DA oxidation can be derived from the sum of the contributions from MAO-A and MAO-B (defined as above). The premise on which this calculation was conducted need not be necessarily true, as pointed out by Fowler et al (1978). However, both Roth (1976) and Donnelly and Murphy (1977) have found the B-form of MAO in the platelet and in the brain to be remarkably similar.

In discussing results obtained from post-mortem tissue it is appropriate to mention the extraordinary stability of MAO. This has been verified many times (see Mackay et al, 1978). Schwartz et al (1974a) for example, found no loss of activity, even after 120h at $5^{o}C$, or after 4h at $33^{o}C$. Furthermore, Owen et al (1979) could detect no difference in activity between human brain biopsy tissue and brain material which had been in the mortuary at $4^{o}C$ for 48h $\pm$ 22h (mean $\pm$ S.D.). These lengths of time all refer to the period over which the intact body was stored; after dissection and homogenization, when the enzyme is disrupted, it is likely to be more labile. Although the method employed in this study, using whole tissue homogenates, has the undoubted advantage of retaining the enzyme, as far as possible in its natural state, variation in just one cellular component, for example in nerve endings, would be diluted by activity from other parts such as glial cells and cell bodies. Student and Edwards (1977) have, in fact, reported that the variation in MAO A/B ratio amongst different rat brain regions is much greater when synaptosomal fractions rather than whole tissue homogenates are compared. In addition, the larger the dissected area, the more likely it is that localized variations in the MAO A/B ratio will be obscured. This was emphasized by Hirano et al (1975) who reported over a 4 fold span in the ratio of 5HT to TYR-oxidising ability in 14 discrete rat hypothalamic areas.

Owen et al (1979) found that brains from two out of six individuals had an anomalous response to deprenyl or clorgyline inhibition. They produced a single sigmoid curve when the percentage inhibition of TYR oxidation was plotted against MAO inhibitor concentration, pointing to the presence of only one enzyme form, as opposed to normal brain tissue which produces a double sigmoid curve, indicating the involvement of two species.

These abnormal brains could, however, still oxidise 5HT and BZ as effectively as those producing the expected pattern of MAO inhibition. No such variation in deprenyl sensitiviy between individuals was apparent in the present study. Owen et al (1979) suggest that events occurring after death, such as the collection and storage of brains, may alter the environment of MAO, in some cases sufficiently to cause uncharacteristic inhibition curves.

Deprenyl only acts as a selective MAO inhibitor within a certain concentration range. Large doses inhibit both forms of the enzyme. Under the in vitro conditions used in this study, 10^{-6}M deprenyl was found to produce optimal selectivity (Figure 5:2). When used clinically in the treatment of Parkinson's disease a dose of 10 mg per day of deprenyl is usually employed which, if evenly distributed in a body water mass of 40Kg, would give a concentration of about 10^{-6}M. Even though this calculation suggests that deprenyl works selectively in vivo, this has yet to be convincingly demonstrated in man. It is also not certain whether DA concentration is raised in the parkinsonian striatum after this dose of deprenyl. It was shown earlier (Chapter 3) that an 80% inhibition of platelet MAO-B is necessary before an increase in unmetabolized PEA can be detected in the urine. In addition, Robinson et al (1973) indicated that an 80% inhibition of platelet MAO needs to be acheived by phenelzine, before this drug is consistently superior to placebo in the treatment of depression. Davidson et al (1978), however, showed that only a 60% inhibition of platelet MAO is necessary for phenelzine to be an effective antidepressant. Green et al (1977) found that only when both forms of MAO are inhibited by about 85% in rat brain can L-dopa produce a change in behaviour or brain DA concentration. In the present study in vitro DA oxidation in the striatal regions was inhibited to the extent of 80-85% by 10^{-6}M deprenyl; it would thus be reasonable to expect that the inactivation of DA by MAO would be impaired in vivo by the doses of deprenyl employed clinically. In fact, post-mortem striatal tissue from parkinsonian subjects who had been receiving 10 mg deprenyl daily inhibited DA oxidation in vitro by about 85% (Riederer et al, 1978). These authors also reported a raised DA concentration in the brain of one parkinsonian subject who had been treated with the unusually large dose of 100 mg deprenyl daily. However, an elevated 5HT

concentration was also detected which indicates that deprenyl does not function as a selective inhibitor at this dose. Riederer et al (1978) also noted that *in vitro* 5HT oxidation was inhibited to the extent of 60-70% by the striatum of patients who had received 10 mg deprenyl daily for 6 days. It is apparent, then, that this dose of deprenyl is not as selective as a 10^{-6}M concentration in the test tube. However, Riederer and Reynolds (1980) have found that this same level of inhibition was present in a subject who had received 10 mg deprenyl daily for 4 years. It is not known, however, whether this degree of suppression of *in vitro* 5 HT oxidation is sufficient to cause an alteration in the concentration of 5HT, or other MAO-A substrates. If it were, it may be important in the interpretation of the beneficial effect of deprenyl in Parkinson's disease. The other major enzyme concerned with DA metabolism, besides MAO, is COMT. When this enzyme methylates DA, 3-methoxytyramine is produced, which in human cortex at least, is a substrate mainly for MAO-A, unlike DA (Roth and Feor, 1978), so that the spread of inhibition to the A form might also tend to prevent the further degradation of DA.

The present results therefore indicate that clinical doses of deprenyl, by virtue of their MAO inhibitory action in human brain, cause DA metabolism to be substantially retarded. It seems likely that, by this mechanism, deprenyl exerts its beneficial effect in Parkinson's disease.

CHAPTER SIX - THE METABOLISM OF DEPRENYL

Introduction

Very few drugs are eliminated from the body entirely unchanged; metabolic transformation typically converts the compound to a more polar one which tends to inactivate it and enables it to be more efficiently excreted. However, there are many cases on record in which metabolism produces the therapeutically-active species or alternatively, instances where toxic products are formed. It is obviously necessary, therefore, to study the metabolism of a drug if one is fully to understand its mechanism of action.

Of all the irreversible MAO inhibitors, most attention has been focussed in the metabolism of those based on hydrazine. They are primarily acetylated and, indeed, the varying ability of individuals to acetylate such drugs is thought by some to affect clinical response (Evans et al, 1965; but see Marshall, 1976).

The metabolism of the non-hydrazine inhibitors has generally received less attention. However, by analogy with pargyline (structure Figure 1:1), which is converted to BZ (Edwards and Blau, 1973; Durden et al, 1976), one might have predicted that deprenyl would be degraded to methamphetamine (Figure 6:1). In fact, a preliminary *in vitro* experiment (K. Blau-unpublished) showed that rat liver homogenate converts deprenyl, to some extent, to methamphetamine and amphetamine. Because of the well-recognized biological activity of these compounds it seemed important to quantify any such conversion to the amphetamines in man after the administration of therapeutically-active doses of deprenyl. Although the GC method used for the determination of PEA (Chapter 2) also detects amphetamines, the pentafluorobenzoyl derivative of methamphetamine does not "electron-capture" well, as previously reported (Matin and Rowland, 1972). However, a GC-MS assay was devised (Reynolds, King, Elsworth, Sandler-in preparation) which enabled methamphetamine and amphetamine to be analysed simultaneously. In addition, unchanged deprenyl in urine and MAO inhibitory substances

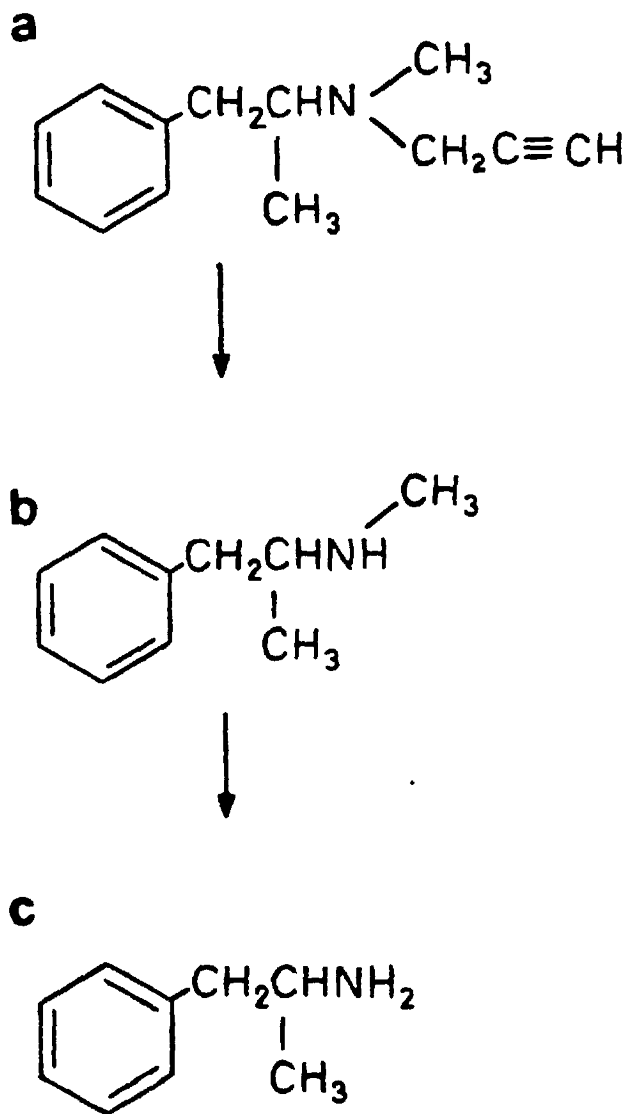


Fig. 6:1 Structures of deprenyl (a), methamphetamine (b) and amphetamine (c).

in plasma were sought.

Once the degradation to methamphetamine and amphetamine had been quantified, it was necessary to determine whether the beneficial effect which some parkinsonian patients had derived from deprenyl were a direct effect or stemmed merely from the action of these metabolites. This was, in fact, a distinct possibility, as amphetamine has recognized anti-parkinsonian properties, first described by Solomon et al (1937) and meticulously confirmed (Parkes et al, 1975).

Two approaches were employed in order to distinguish between the clinical effects of MAO inhibition and that due to amphetamines. The urinary excretion of basic compounds such as methamphetamine and amphetamine is a pH-dependent process (Beckett and Rowland, 1965a, b). Passive reabsorption of unionized drug occurs in the kidney so that, for example, under acidic conditions when relatively largely ionized, the compound is quickly cleared from the body. Thus, the urinary pH was deliberated altered in a group responding favourably to deprenyl to determine whether varying the elimination rate of the amphetamines affected the patient's disability. The problem was also tacked more directly by actually substituting deprenyl for an equivalent amount of methamphetamine and amphetamine.

Materials

Optical isomers of amphetamine and (+)-methamphetamine were gifts from Smith, Kline and French, Ltd., Welwyn Garden City, U.K.

(-)-Methamphetamine was synthesized from (-)-amphetamine by Dr. B.L. Goodwin.

Methods

(i) Determination of methamphetamine and amphetamine

Paired urine samples from subjects taking part in a double-blind crossover study on the effect of deprenyl on sleep were used to quantify the conversion of the drug to methamphetamine and amphetamine. When receiving deprenyl, 4 subjects took 10 mg and 2 subjects 5 mg for 3 days. 24 h

urine collections were made on the final day.

Extraction and derivatization procedures are the same as those used for "total" PEA assay (Chapter 2), except that 0.5 ml urine was used. However, instead of the pentafluorobenzamides being analyzed by electron-capture GC, they were detected by single-ion monitoring on a GC-MS (LKB 9000S) assembly. This manoeuvre was necessary because of the low electron-capturing ability of methamphetamine pentafluorobenzamide (Table 6:1). TEA was used as internal standard which, in common with the amphetamines, produces an m/e 118 fragment, corresponding specifically to methyl-substituted phenylethyl moieties.

(ii) Deprenyl assay

To 5 ml urine from subjects who had received deprenyl was added 2 M NaOH until the pH was in excess of 12. Diethyl ether (20 ml) was added and the mixture shaken for 5 min. The ether layer was separated and its contents back-extracted into 2 ml 1 M HCl by shaking for 5 min. The aqueous phase was removed and its pH raised above 12 with 5 M NaOH. It was then re-extracted with 10 ml ether by vortex mixing for 30 sec. After briefly centrifuging (1000g for 1 min), the ether layer was separated and its volume reduced under a stream of nitrogen. This ether solution was analyzed by GC-MS. A 6 ft 3% PPE-21 column was used at $200^{o}C$ with a helium carrier gas flow of 50 ml min^{-1}. The two major ions found to be associated with the mass spectrum of deprenyl (m/e 56 and 96) were monitored. The method proved sensitive enough to detect 10 ng ml^{-1} authentic deprenyl.

(iii) Plasma MAO inhibitor assay

The effect of normal plasma on rat liver MAO activity was compared with plasma obtained from the same subject following the ingestion of 10 mg deprenyl 2 h earlier. The MAO assay method used was that described in Chapter 2, except that 50 µl of plasma was the inhibitor solution and the reaction was terminated with 0.2 ml 2 M citric acid. A 5% rat liver homogenate was the enzyme source and PEA (125 µM) the substrate. Assuming a minimum of between 10^{-7} and 10^{-8} M deprenyl could be detected in this fashion (Fig. 3:6), this technique has a

Table 6:1

Comparative electron-capturing ability of pentafluorobenzamides

Amine	Response relative to TEA*
PEA	1.39
Amphetamine	1.00
Methamphetamine	0.03

*TEA is the internal standard.
The responses were obtained using a nickel-63 detector and chromatographic conditions as described for PEA determination (Chapter 2). The results agree with those of Matin and Rowland (1972).

sensitivity limit of about 10 ng ml^{-1}.

(iv) Alteration of urinary pH

Five patients with idiopathic Parkinson's disease, responding favourably to the addition of deprenyl to their L-dopa therapy, agreed to participate in the study, the objects of which were carefully explained to them. The duration and severity of disease was similar in all subjects. The trial was carried out in double-blind fashion, and involved collection of three 24 h urine samples, one after each of the the following 3 day treatment phases (a) placebo (b) 4 g ammonium chloride daily (c) 6 g sodium bicarbonate daily. Preparations were dispensed in indistinguishable capsules. The volume and pH of the urine was measured and an aliquot stored at $-20^{o}C$ until assay.

Parkinsonian disability was scored at the end of each stage, patient diaries being used where oscillations were predominant. These volunteers also completed visual analogue mood rating scales at each stage of the study.

Each phase of the trial was separated by between 4 and 16 days.

(v) Substitution of deprenyl for methamphetamine and amphetamine

Only 4 of the 6 patients who entered this study completed all 3 phases. Three of these 4 subjects also took part in the experiment (iv) described above. All were receiving benefit from the addition of deprenyl to their L-dopa therapy. In a double-blind manner, deprenyl was substituted for the following preparations, identically presented for three weeks each (a) two placebo capsules containing lactose (b) two capsules each containing 4 mg (-)-methamphetamine and 1 mg (-)-amphetamine and (c) two 5 mg capsules of deprenyl itself.

The disability of the patients was assessed weekly and, in the case of those experiencing oscillations, their diaries scored also.

Results

(i) GC-MS assay for methamphetamine and amphetamine

Originally, the identity of methamphetamine and amphetamine was confirmed by comparing the mass spectra of these compounds in urine to authentic substances derivatized in the same manner. For routine estimations the ion of m/e 118 was monitored, for which typical standard and specimen traces are shown in Fig. 6:2. Good resolution and absence of interfering material is evident.

The accuracy and precision of the method were also satisfactory. Six normal urine specimens, each "spiked" with 500 ng methamphetamine and TEA were calculated to contain 508 ± 49 (S.D.) ng methamphetamine and 499 ± 23 ng amphetamine. As little as 1 ng ml^{-1} of these substances in the original specimen could be quantified.

(ii) Quantification of deprenyl metabolism

The urinary output data for the excretion of methamphetamine and amphetamine after deprenyl administration are shown in Table 6:2. Large quantities of methamphetamine and substantially less amphetamine were consistently found. Neither compound was present in the samples collected whilst subjects were taking placebo. Authentic deprenyl (0.02 mg in 0.5 ml H_2O) taken through the entire procedure did not produce any amphetamines.

Although, in this set of results, a statistically significant correlation did not exist between urinary pH and excretion of the amines, the output of methamphetamine was greatest in the two most acidic specimens.

Whilst the GC-MS search for unmetabolized deprenyl proved negative, there was a significant decrease ($p<0.05$, paired t test) in the MAO activity of liver when incubated with plasma from subjects who had received deprenyl 2 h earlier, compared with baseline level (Table 6:3).

(iii) The contribution of deprenyl metabolites to its therapeutic action

The co-administration of either sodium bicarbonate or

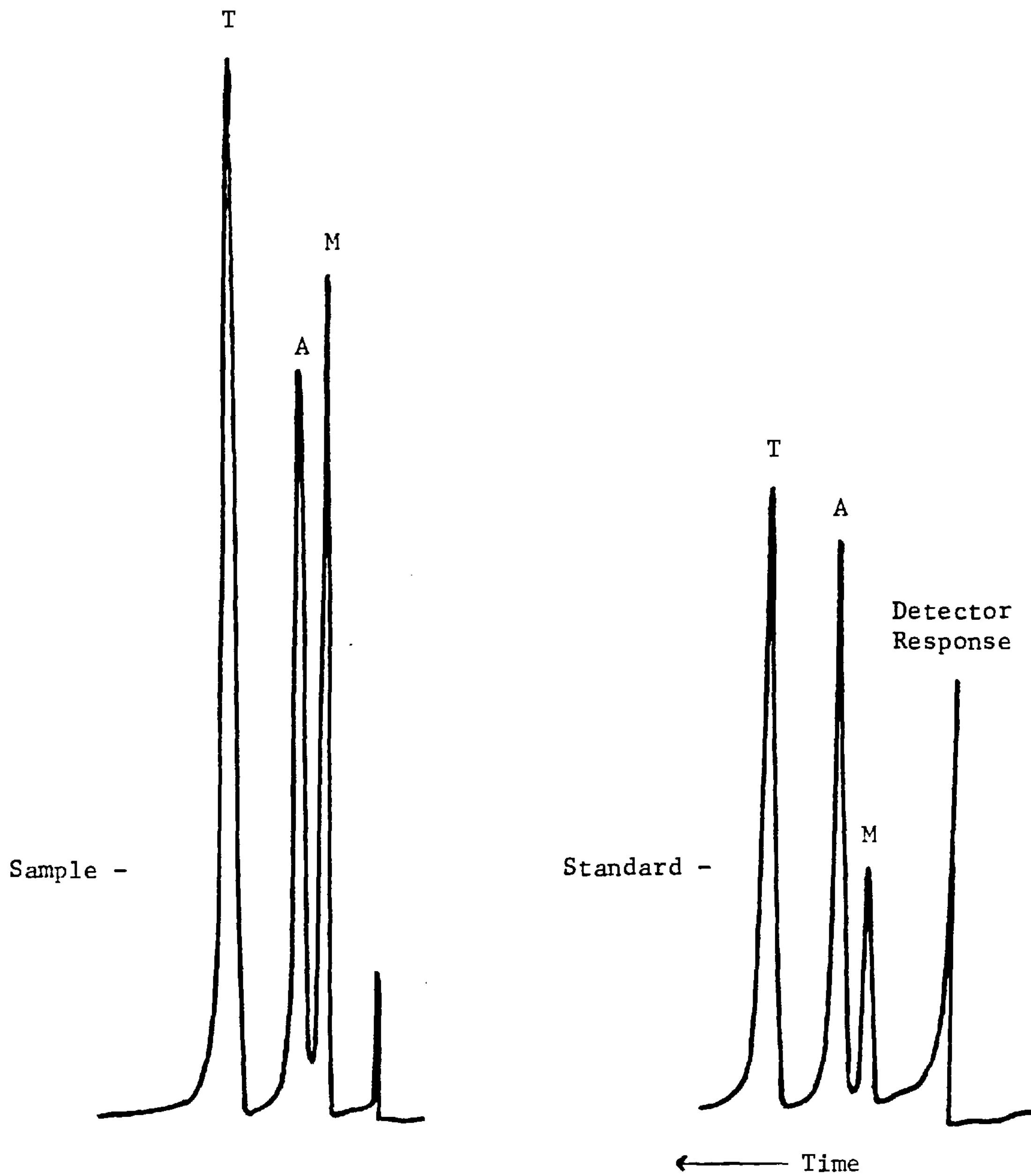


Fig. 6:2 Showing typical traces obtained in the GC-MS assay of methampetamine and amphetamine.

Methamphetamine (M), amphetamine (A), and internal standard (T) peaks in a standard and a urine sample (at different attenuation values).

Table 6:2 Urinary output of methamphetamine and amphetamine in normal subjects

Subject No	Deprenyl HCl (mg per day)	Urine pH	Urine Vol (ml)	Amphetamine excretion (mg)	Amphetamine excretion (equivalent % dose)	Methamphetamine excretion (mg)	Methamphetamine excretion (equivalent % dose)
1	5	6.4	1435	0.48	15.8	1.67	50.2
2	5	5.9	1400	0.59	19.5	3.47	104.0
3	10	6.1	2100	1.05	17.3	3.42	51.3
4	10	6.4	1190	0.88	14.5	3.55	53.2
5	10	5.8	1280	0.67	11.1	4.98	74.6
6	10	6.8	1600	0.74	12.2	3.08	46.2

Table 6:3

The presence of a MAO inhibitory substance in plasma after deprenyl administration

Subject	Liver MAO activity with added plasma (cpm x 10^3)	
	Baseline	After 10 mg deprenyl
1	19.43	17.29
2	18.04	17.93
3	17.87	14.95
4	17.70	16.48
5	17.30	16.02

ammonium chloride with deprenyl and L-dopa therapy did not alter the disability of parkinsonian volunteers, neither did any of the patients report any mood changes.

However, urine collections made during these switches showed that ammonium chloride and sodium bicarbonate had the expected effect on the excretion of methamphetamine and amphetamine (Table 6:4). There was a highly significant correlation with pH in the excretion of amphetamine ($r=0.82$, $p<0.001$) and methamphetamine ($r=0.84$, $p<0.001$).

When uncontrolled, the mean urinary pH was 6.44, and methamphetamine excretion represented on average 43.54% of the deprenyl dose whereas amphetamine output contributed 15.14%. Treatment with sodium bicarbonate elevated the mean urinary pH to 7.20 and in this instance the mean methamphetamine excretion was equivalent to 15.44% of the dose and amphetamine output 6.76%. Ammonium chloride administration resulted in a mean urinary pH of 5.50. Mean methamphetamine and amphetamine excretion was equivalent to 51.68% and 17.39% of the dose of deprenyl respectively.

In the methamphetamine-amphetamine substitution study, 2 of the 4 patients (both oscillators) deteriorated during the amphetamine and placebo phases, but not during the period when they were receiving deprenyl. However, the other 2 subjects (one oscillator) remained unchanged throughout the study.

Discussion

When synthesizing deprenyl, Knoll et al (1965) deliberately intended it to possess "both the amphetamine-like psychostimulant effect and the psychoenergetic effect of the potent MAO inhibitors." That it would actually be metabolized to methamphetamine and amphetamine was not foreseen.

However, Tables 6:2 and 6:3 show that urinary methamphetamine accounts for the major portion of an ingested dose of deprenyl, with amphetamine contributing substantially less. The corresponding conversion of pargyline to benzylamine has not been quantified (Durden et al, 1976); nor has the breakdown of tranylcypromine which, it appears, is also metabolized to some extent to methamphetamine and amphetamine

Table 6:4

Effect of urinary pH on methamphetamine and amphetamine excretion in parkinsonian patients

Subject No	Deprenyl HCl (mg per day)	Treatment *	Urine pH	Urine Vol (ml)	Amphetamine excretion (mg)	Amphetamine excretion (equivalent % dose)	Methamphetamine excretion (mg)	Methamphetamine excretion (equivalent % dose)
1	5	U	6.5	2460	0.50	16.53	1.51	45.28
		A	5.6	2200	0.51	16.53	1.68	50.37
		B	7.0	2900	0.37	12.23	0.85	25.49
2	7.5	U	6.4	1500	0.96	21.16	3.16	63.17
		A	5.2	1700	1.11	24.46	2.95	58.97
		B	7.2	1200	0.34	7.49	0.97	19.39
3	10	U	6.8	1060	0.31	5.12	0.76	11.39
		A	5.8	1720	0.82	13.55	1.91	28.64
		B	7.2	1580	0.41	6.78	0.59	8.85
4	15	U	6.5	1200	0.96	10.58	3.61	36.08
		A	5.9	1200	1.07	11.79	5.15	51.47
		B	7.2	1100	0.37	4.08	1.71	17.09
5	45	U	6.0	1140	6.08	22.33	18.55	61.80
		A	5.0	900	5.61	20.61	20.19	68.93
		B	7.4	850	0.88	3.23	1.92	6.40

*Urine pH either uncontrolled (U) or rendered acidic (A) or alkaline (B).

(Youdim et al, 1979).

The data (Tables 6:2 and 6:3) also indicate the importance of urinary pH in the excretion of the amphetamines (Beckett and Rowland, 1965a, b). In the experiments where parkinsonian patients were given acidic and basic drugs, an approximately 3-fold change in the excretion rate of methamphetamine and amphetamine occurred. In fact, the anomalously high concentration of urinary methamphetamine in normal subject 2 is explicable in terms of an unusually alkaline urine the previous day causing retention of much of the methamphetamine, which was then excreted the following day in a fairly acidic urine. Beckett and Rowland (1965a, c) reported that ammonium chloride treatment in 3 human volunteers caused 55-70% of an oral dose of (+)-methamphetamine to be excreted in 16 h compared with only 0.6-2% after sodium bicarbonate administration. They also found that methamphetamine was excreted largely unchanged, with a small amount being N-demethylated to amphetamine. The proportions of these two amines measured after deprenyl in the present study are similar to those found by Beckett and Rowland (1965a, c) after a dose of methamphetamine itself. This is not surprising as no unchanged deprenyl could be detected in the urine, so that it seems likely that the inhibitor is completely degraded to methamphetamine, which is subsequently dealkylated to some extent to amphetamine. The small but significant MAO-inhibitory capacity of plasma taken from subjects just 2 h after deprenyl is probably due to traces of unmetabolized drug. Although the amphetamines do inhibit MAO themselves (Miller and Clarke, 1978), the concentration required is higher than could have been derived from the dose of deprenyl, unless it had been concentrated at a particular site. In addition, their inhibitory effects are reversible and are more pronounced against MAO-A than MAO-B so that they would not have been detected in the assay procedure used. The level of inhibition exerted by plasma from the treated subjects on liver MAO activity probably represents about 1% unchanged deprenyl.

Not all of the deprenyl administered was accounted for by methamphetamine and amphetamine. Presumably the remainder is represented by other metabolites derived from methamphetamine. Being one of the most studied drugs in pharmacology, the metabolism of amphetamine-like compounds has been extensively investigated and is well reviewed by Caldwell (1976). However, important species differences exist in this area and the literature concerning metabolism is less comprehensive in man than in experimental animals. A clue to the fate of the remaining portion of deprenyl was, however, provided by Caldwell et al (1972). They found that although racemic methamphetamine administered to human volunteers is mainly excreted unchanged, significant quantities of ring hydroxylated metabolites and amphetamine are present.

With the exception of β-hydroxylation, the liver is the principal site for all the various metabolic reactions of the amphetamines (Caldwell, 1976). Differences do exist, however, in the way the optical isomers are handled by the body, and in man the (+)-isomer is metabolized more rapidly than the (-)-form (Wan et al, 1978; Beckett and Rowland, 1965a, b, c). Although the optical rotation of the product of a reaction is not necessarily that of the original substance, in the case of (-)-deprenyl there is experimental evidence that the (-)-isomers of the amphetamines are generated. (-)-Deprenyl is a much more potent inhibitor of MAO than the (+)-form (Knoll and Magyar, 1972) and the former is now used alone in clinical practice. However (+)-deprenyl was shown by Magyar et al (1967) to have considerably greater amphetamine-like action than (-)-deprenyl, and despite the report of Coyle and Snyder (1969b) that the amphetamine isomers are equivalent at inhibiting DA uptake, more recent work has indicated that the (+)-form is more effective in both inhibition of reuptake and stimulation of release of DA and NA (Harris and Baldessarini, 1973; Holmes and Rutledge, 1976).

That it is the (-)-amphetamine isomers which are generated is supported by the notable absence of any of the

typical CNS effects of amphetamine in subjects receiving (-)-deprenyl. The central action of (+)-amphetamine is 3-4 times larger than that of the (-)-isomer (Innes and Nickerson, 1975). Indeed, Beckett and Rowland (1965c) noted that the administration of 11 mg (-)-methamphetamine base caused no subjective changes whatsoever in human volunteers, in contrast to an identical dose of (+)-methamphetamine. Further, in a double-blind crossover study on the effect of 5 or 10 mg (-)-deprenyl on human sleep, an assessment of mood (visual analogue rating scales) was undertaken which revealed no changes which could be attributed to amphetamine (Thornton, Doré, Elsworth, Herbert, Stern-submitted for publication). However, in its use as an adjuvant to L-dopa therapy in Parkinson's disease, (-)-deprenyl has now been employed in high doses, and it is noteworthy that at a level of about 40 mg daily, some amphetamine-like effects have been observed (Stern et al, 1978). With low doses of deprenyl, the amphetamine-like effects which one might expect to become significant are not those to which tolerance develops, but rather those which progressively increase with continuing treatment, e.g., hyperactivity and stereotypy in animals (Segal and Mandell, 1974; Klawans and Margolin, 1975; Severs et al, 1976). It is interesting that the activity of neurones in the caudate-putamen, regions affected in Parkinson's disease, display this progressive augmentation in response to prolonged amphetamine administration in animals (Rebec and Groves, 1976). However, the two clinical trials reported in this chapter involving manipulation of urinary pH and the substitution of deprenyl for methamphetamine and amphetamine, taken together, indicate that these metabolites of the drug do not play a role in the beneficial effect of deprenyl in Parkinson's disease.

Cavanaugh et al (1970) reported that chronic amphetamine administration in man reduces the pressor response to TYR. Thus, it is conceivable that the absence of the "cheese effect" following deprenyl treatment (Chapter 3) stems from the metabolites of the drug. However, this aspect requires further

study as extremely large doses of (+)-amphetamine were employed, the subjects all had a history of drug abuse and, interestingly, acute amphetamine administration resulted in an increased sensitivity to TYR.

In instances where deprenyl is employed in large doses, as is often the case in animal experiments, one should be aware of the presence of the amphetamine metabolites. In fact, Simpson (1978) working in the rat, reported that (-)-deprenyl acts as an indirectly acting sympathomimetic amine, a finding which can now be explained in terms of pharmacologically-active metabolites. Also of interest are the experiments in the unilateral 6-hydroxydopamine nigrotomized rat model, in which deprenyl produces a mild ipsilateral rotation "of the type seen with amphetamine" (Yahr, 1978).

In conclusion, it appears that despite the anti-parkinsonian properties of (-)-amphetamine (Parkes et al, 1975), the usefulness of deprenyl in the treatment of this disease does not totally depend on the generation of pharmacologically-active metabolites. However, these patients typically receive deprenyl daily for long periods of time, so that a careful watch should be kept for signs of amphetamine toxicity by a reverse-tolerance effect (Klawans and Margolin, 1975; Severs et al, 1976) or even abuse. Psychosis following ingestion of very low doses of amphetamine occurs in certain susceptible subjects (Young and Scoville, 1938), a syndrome which resembles paranoid schizophrenia in many respects (Cannon, 1977). Such adverse reactions can probably be minimized by maintaining a maximal acid urine so as to facilitate the excretion of these substances.

There is one final point, this study may well provide a good pointer to the way the body will handle a large number of propargylamine MAO inhibitors structurally related to deprenyl, recently synthesized by Knoll et al (1978).

CHAPTER SEVEN - TMH AS A MAO-B SUBSTRATE IN MAN

(i) General introduction

HA is widely distributed throughout the animal kingdom, but in general is present in particularly high concentration in the lungs, intestinal mucosa, and skin (Reite, 1972; Douglas, 1975). In many tissues HA is stored in mast cells where its turnover is slow; it may take a matter of weeks to replenish depleted stores. However, in sites where HA is not stored in mast cells, such as the intestinal mucosa and the greater proportion of the brain it undergoes rapid turnover (Douglas, 1975).

In the periphery, HA participates in several important physiological processes, notably anaphylaxis, allergy, injury, shock and gastric secretion (Douglas, 1975). Its effects are mediated via two distinct populations of receptors called H_1 and H_2 (Black et al, 1972) each of which is refractory to antagonists of the other.

(ii) Histamine as a central neurotransmitter

In the brain, the function of HA is far from certain, but there is a large body of evidence which points to it being involved in neurotransmission. Reviews dealing specifically with this aspect have been published (Green et al, 1978; Schwartz, 1977; Calcutt, 1976). The evidence will, therefore, be briefly summarised.

HA has a non-uniform distribution in the brain, concentrations in the midbrain being consistently high in all species. The hypothalamus is invariably the richest source of HA, usually about 1 µg/g wet weight (Green, 1970); but even its distribution within the hypothalamus has been shown to be uneven (Brownstein et al, 1974).

No suitable histochemical method exists for visualising HA-containing nerves but the technique of measuring the amine and the enzyme responsible for its synthesis chemically, caudally and distally to lesions, led to the suggestion (Schwartz et al, 1979) that ascending histaminergic fibres arise

from the posterior hypothalamus or upper midbrain. Schwartz et al also put forward evidence pointing to the existence of a descending pathway innervating the brainstem.

Studies employing subcellular fractionation showed that a proportion of HA in the brain is present in synaptosomes and, furthermore, that it is associated with synaptic vesicles (Kataoka and DeRobertis, 1967) within this anatomical structure.

Reserpine has been shown to deplete central HA stores. In the rat hypothalamus, for example, it brought about an increase in disappearance of ^{3}H-HA _in vivo_, together with an increased rate of synthesis (Pollard et al, 1973). In an _in vitro_ preparation of the same rat brain area, reserpine caused a release of HA, but with an accompanying decrease in synthesis (Verdière et al, 1974). This discrepency might have arisen because the _in vitro_ hypothalamus is isolated from its normal connections with other areas.

Release of HA following nerve stimulation has not been reported. However, potassium-evoked depolarisation has been shown to increase the release of endogenous HA _in vitro_ in a calcium dependent manner (Taylor and Snyder, 1972). Iontophoretically applied HA depressed or excited the firing of cat cortical neurones (Phillis et al, 1968) whilst those of hypothalamus are excited (Haas, 1974).

HA was found to be one of the most potent stimulators of cyclic 3', 5' AMP-monophosphate, an effect potentiated by a phosphodiesterase inhibitor (Kakiuchi and Rall, 1968).

Peripheral loading with HIS produced an elevation in brain HA levels, whilst maintaining the normal pattern of distribution, but these changes failed to cause behavioural alteration in the rabbit (Abou et al, 1973), unlike the rat (Maśliński et al, 1973). In the latter study, catalepsy and rearing were noted. However the CNS function most convincing connected with HA is that of fluid balance control. HA injected intracerebroventricularly produced an increase in water intake in satiated rats, which was antagonised by H_1-receptor blocking drugs (Gerald and Maickel, 1972). In the

cat the release of ADH and its consequent antidiuretic effect has been attributed to an H_1 receptor-stimulating action of HA (Bennett and Pert, 1974).

Enzymes for the metabolism and catabolism of HA are present in the brain, their distribution roughly paralleling that of HA itself (see below).

Thus, despite much of the evidence being circumstantial and without all the criteria for acceptance as a transmitter being fulfilled, HA is well worthy of consideration for a candidate neurotransmitter role.

The rest of this section will be devoted to the metabolism of HA in the CNS and the importance of its methylated metabolite TMH (tele-methylhistamine, also called 1-methyl-4-β aminoethylimidazole; 1, 4-methylhistamine). For discussion of terminology, see Black and Ganellin (1974).

(iii) Histamine metabolism

As HA does not readily pass the blood-brain barrier (Snyder et al, 1966), ingested or peripheral HA is unlikely to contribute significantly to the central store. In fact, every tissue which contains HA appears capable of synthesizing it from the essential amino-acid, HIS. Both the non-specific AAAD and the "specific" HD are able to decarboxylate HIS. Schwartz et al (1970) studied the kinetics of HA in homogenates from rat hypothalamus and the effects of selective inhibitors of HD and AAAD and concluded that HD is only enzyme involved in HIS decarboxylation. This finding permitted the extrapolation that HA is not formed in serotonergic or catecholaminergic nerves, a suggestion which received support when it was shown that chemical lesions induced by 6-hydroxydopamine left HD unaltered (Garbarg and Barbin, 1976). Subcellular fractionation studies showed that the major portion of rat brain HD is located in the cytoplasm of nerve endings (Baudry et al, 1973a).

Pathways for the catabolism of HA are shown in Figure 7:1.

The acetylation of HA is a very minor metabolic route in mammals, and has not been shown to occur centrally. Traces of labelled N-acetylhistamine have been identified in human

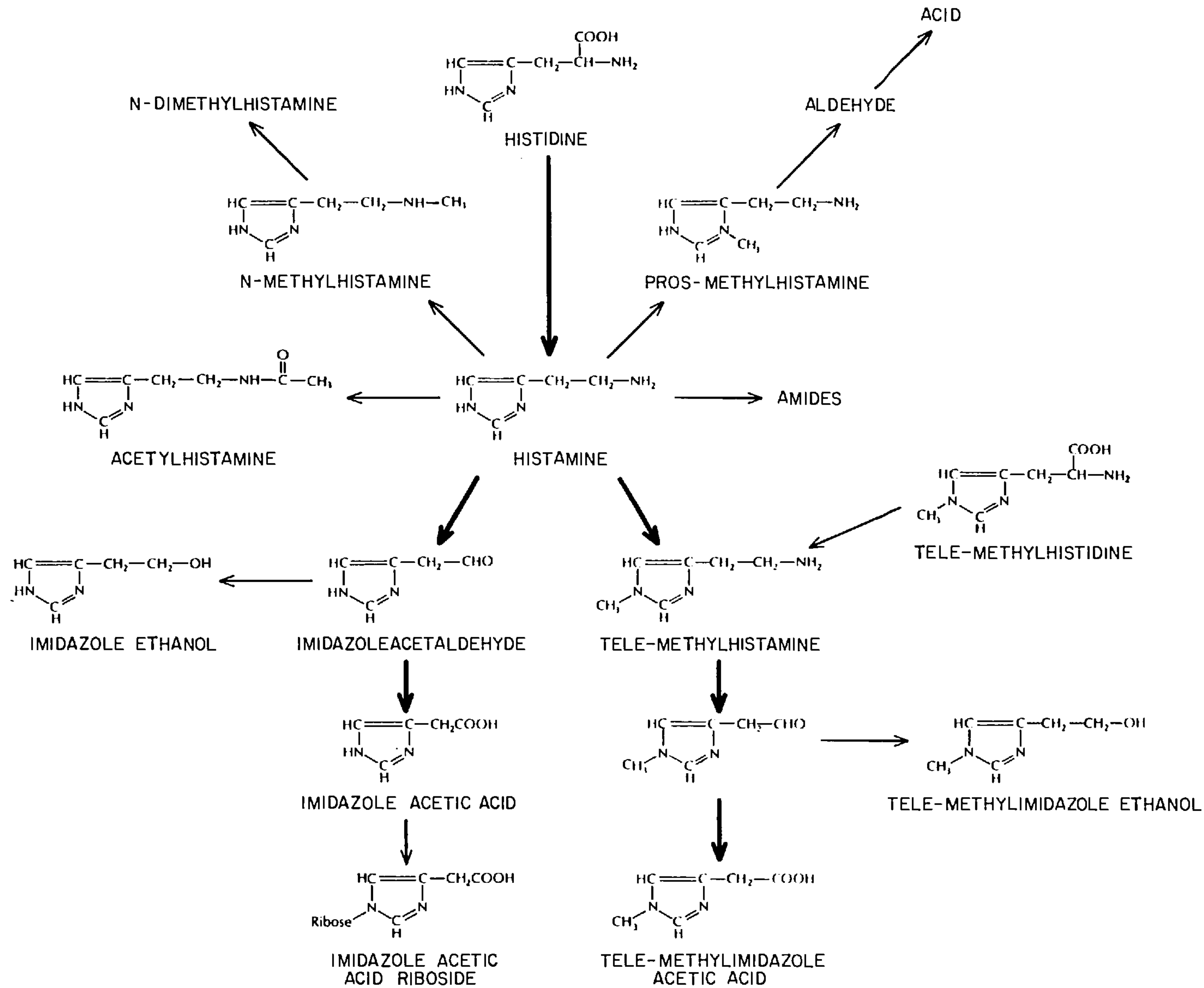


Fig. 7:1 Pathways of histamine metabolism Thick arrows indicate major routes

urine after the administration of labelled HA (Schayer and Cooper, 1956).

Methylation of the side chain nitrogen to form N-methylhistamine or N, N-dimethylhistamine has not been rigorously shown to occur in mammals. Although N-methylhistamine was found in normal human urine (Kapeller-Alder and Iggo, 1957), the possibility exists that it arose from bacterial metabolism of HA.

After very large doses of HA, methylation of the ring nitrogen closest to the side chain of HA has been described in man (Karjala and Turnquest, 1955). This compound, _pros_-methylhistamine (1, 5-methylhistamine), is then converted to the corresponding acid.

HA is capable of forming amides with palmitic and probably other fatty acids, and this reaction is catalysed by the microsomal fraction of liver (Bachur and Udenfriend, 1966). The amide has not yet been looked for in other tissues though.

HA is not a substrate for MAO (Oreland, 1972), but it is deaminated by diamine oxidase (histaminase) to form imidazole acetaldehyde, most of which is converted by aldehyde dehydrogenase to IAA. 25 to 34% of a physiological dose of ^{14}C-HA injected intradermally into man was recovered in the urine as IAA or IAA conjugated with ribose (Schayer and Cooper, 1956). Only 2 to 3% was recovered as unchanged ^{14}C-HA, so the major proportion, 46-55%, comprised metabolites, formed after methylation of the ring nitrogen, TMH and MIAA. One factor which might explain the incomplete retrieval of the radiolabel is that other unidentified metabolites were formed. IAA might conceivably undergo extensive ring degradation, as it does in some micro-organisms (Hayaishi et al, 1954). The acetaldehydes could participate in some non-oxidative reactions. In fact, the reduction of imidazoleacetaldehyde to form imidazole ethanol (histidol) could well be a minor pathway of catabolism, as small amounts of this product have been found in the urine of man and rat after treatment with an aldehyde dehydrogenase inhibitor (Nakajima and Sano, 1964).

Furthermore, not all of an administered dose of ^{14}C-TMH given to mice could be accounted for either as unchanged amine or as ^{14}C-MIAA (Rothschild and Schayer, 1958).

Methylation of the ring nitrogen of HA, therefore, is its major route of metabolism. In fact, in mammalian brain, where diamine oxidase is absent (Burkard et al, 1963), it is perhaps the only route. An early study (Snyder et al, 1966) did, however, report the formation of ^{3}H-IAA from ^{3}H-HA in rat brain, but subsequent research did not confirm this result in mouse, or rat brain (Schayer and Reilly, 1973; Schwartz et al, 1971). Indeed, labelled TMH is rapidly found in brain after the administration of labelled HA (Schwartz et al, 1971; Knight and Smith, 1979) or labelled HIS (Schayer and Reilly, 1973; Pollard et al, 1974).

HMT is a highly specific enzyme which has a regional and subcellular distribution in brain resembling that of HA (Snyder et al, 1974). A recent study showed that there was a statistically significant correlation between HA and TMH in discrete regions of rat brain (Hough and Domino, 1979a).

(iv) The importance of TMH

As TMH lacks the characteristic activities of HA on brain tissue, it has been stated that formation of the former must merely be an inactivation process (Schwartz, 1977). However, as Green (1964) has pointed out, it and other HA metabolites could be active on systems not yet tested. Methylation of several naturally occurring compounds results in an active molecule, albeit with qualitatively different actions from the parent substance. Melatonin and A are, for example, methylated derivatives of 5HT and NA respectively. Even though TMH has never been found to be more potent than HA, it was reported to have an equipotent effect on the firing of cortical neurones when applied iontophoretically (Phillis et al, 1968), and to exhibit some of the properties of HA on brainstem neurones (Haas et al, 1973). TMH accumulates after treatment with MAO inhibitors, in the urine of patients taking isocarboxazid (Fram and Green, 1968), in cat brain after iproniazid (White, 1966) and in rat brain following tranylcypromine (Schwartz et al, 1971), pargyline or deprenyl (Hough and Domino, 1979b). MAO, therefore, seems

responsible for the deamination of TMH to the aldehyde which is then converted to MIAA. Although TMH might theoretically be a substrate for diamine oxidase also, experiments on the effect of an inhibitor of this enzyme and an inhibitor of MAO on the conversion of TMH to MIAA in mice concluded that diamine oxidase plays little or no part in the production of MIAA (Rothschild and Schayer, 1958). This study also found no evidence of any hydrolysable conjugates of TMH in urine, and recently (Knight and Smith, 1979) could not detect any demethylation of TMH to HA in rat brain.

Another fact which could be taken as a pointer to the importance of TMH is the existence of a pathway capable of generating it without the transmethylation of HA (Schwartz et al, 1973). In this study several mouse tissues, notably those rich in HD, were able to form ^{3}H-TMH from ^{3}H-*tele*-methylhistidine. Further *in vivo* work employing HD and AAAD inhibitors indicated that the "specific" HD was responsible for the decarboxylation step. This was confirmed by *in vitro* kinetic studies which showed substrate competition between ^{3}H-HIS and ^{3}H-*tele*-methylhistidine. The latter was converted less readily, however, to the corresponding amine. This study also showed the release of ^{3}H-TMH synthesized from ^{3}H-*tele*-methylhistidine, *in vivo* from mouse stomach and *in vitro* from rat peritoneal mast cells, by treatments known to elicit HA release. This finding indicates that TMH was being held in the same cellular stores as HA.

With regard to the localization of TMH, there is on the one hand evidence that it and HMT are associated with the mitochondrial fraction of the brain (Green, 1970) whilst, on the other hand, Waldmeier et al (1977) emphasised that exogenous and endogenous HA are metabolized similarly, without there being an identified uptake mechanism for HA, so that HA must be methylated extraneuronally. These two pieces of evidence need not be contradictory, however; the enzyme could be present intra- and extra-neuronally. In fact, HMT has been identified in cultured glial cells (Garbarg et al, 1975).

It is also of interest that TMH is a potent inhibitor of HMT *in vivo* and *in vitro* (Schayer and Reilly, 1973). SAH formed from SAM, the methyl donor required by HMT, also inhibits HMT (Zappia et al, 1969). So TMH and SAH might regulate the activity of HMT and thereby affect the concentration of HA. Another transmethylating enzyme, COMT, similarly requires the presence of SAM and is inhibited by SAH. Baudry et al (1973b) found that administration of certain COMT substrates to guinea-pigs increased SAH production sufficiently to inhibit the methylation of intracisternally injected ^{3}H-HA. This indicates that important interactions between catecholaminergic systems and the putative histaminergic systems might occur.

Despite the regulatory effect which TMH can exert on HMT, administration of MAO inhibitors cause large increases in brain TMH concentration. One explanation for this, put forward by Hough and Domino (1976b) is that if TMH is formed in a different cellular compartment to that in which it is oxidised, then TMH accumulation after MAO inhibition might not be of sufficient magnitude, or in the proper location to inhibit its own formation. Another possibility is that the production of TMH from *tele*-methylhistidine occurs in a separate compartment and this is largely responsible for the raised TMH concentration detected after MAO inhibition.

Therefore, whether TMH is important in its own right or whether it can indirectly regulate HA or other amine concentrations, it is important to ascertain more precisely the nature of its catabolism.

(v) TMH as a substrate for MAO

Interest in the role of MAO in the oxidation of TMH was initiated by Waldmeier et al (1977). This group showed that the MAO-B inhibitor, deprenyl and not the MAO-A inhibitor, clorgyline caused an elevation of ^{3}H-TMH and a decrease of ^{3}H-MIAA after intracisternal injection of ^{3}H-HA into rat brain. They concluded that TMH is metabolized by MAO-B in rat brain and furthermore suggested that TMH may be a more specific substrate for MAO-B than PEA. This suggestion arose because

clorgyline, at doses which affected PEA deamination *in vitro* did not alter ^{3}H-TMH metabolism *in vivo*. However, from examination of their data, it seems possible that the degree of inhibition of PEA oxidation recorded *in vitro* would not have been sufficient to change its metabolism *in vivo*.

Whilst the present investigation on TMH oxidation was being carried out, Hough and Domino (1979b) reported their results on the subject. They found an IC_{50} value of 4×10^{-7} M for deprenyl on TMH oxidation in rat hypothalamus, which was similar to 2.6×10^{-7} M which they obtained for deprenyl on PEA oxidation in whole brain of the rat. Their thermostability studies indicated that the susceptibility of inactivation of TMH is similar, but not identical, to that of PEA. They also performed some *in vivo* work, which indicated that deprenyl, but not clorgyline, affects rat whole brain TMH concentration. Taken overall, the contributions of Hough and Domino (1979b) and Waldmeier et al (1977) suggest that MAO-B is responsible for TMH oxidation, but do not exclude the possibility of an enzyme different from MAO-B, but also sensitive to deprenyl, being involved.

The present study examined the oxidation of TMH by human, as opposed to rat, tissue. Liver was chosen as this organ, which is a rich source of MAO-B as well as of MAO-A. The sensitivity of TMH and PEA oxidation to deprenyl were examined, and the specific activities of the reaction towards PEA and 5HT compared. Substrate competition experiments between PEA or 5HT and TMH were also performed, to determine whether TMH and PEA are metabolized by the same enzyme form and also to assess the affinity of TMH for the enzyme.

Materials

TLC sheets-plastic, coated with F1440 cellulose
Schleicher and Schüll-Dassel, W. Germany
^{3}H-TMH-Dr. L. Maître, Ciba-Geigy Basle, Switzerland
TMH, MIAA-Calbiochem, San Diego, U.S.A.
^{14}C-PEA-New England Nuclear, Boston, U.S.A.
^{14}C-5HT-Radiochemical Centre, Amersham, U.K.
Instagel-Packard Instruments, Caversham, U.K.

Methods

(i) Direct assay of ^{3}H-TMH oxidation

A chromatographic system similar to that used by Waldmeier et al (1977) was employed to separate substrate from product. TLC sheets were run in a solution of chloroform: methanol: 25% ammonia (12:7:1) and visualisation of spots was achieved by iodine vapour. ^{3}H-TMH was purified before use by adding cold TMH (500 μM) and running on the above system. The band corresponding to a cold marker spot of TMH was eluted with 100 mM phosphate buffer, pH 7.4.

The reaction mixture for the enzyme assay comprised 10 μl of a 15% $^{w}/v$ human liver homogenate in 10 mM phosphate buffer pH 7.4, 3 μl H_2O (or 10^{-2} M deprenyl for blanks), and 20 μl of the purified ^{3}H-TMH. The concentration of ^{3}TMH added was approximately 500 μM as the substance was eluted from the cellulose in the same volume in which it was applied. Based on this assumption, the specific activity was 180 μCi/mmol. This was incubated for 2 h at 37°C. The mixture was then applied to a TLC sheet and ran as described above. Consecutive 1 cm sections of the plate were eluted and counted.

(ii) The effect of deprenyl on ^{3}H-TMH oxidation

For the determination of deprenyl sensitivity, 10 μl of the 15% $^{w}/v$ liver homogenate was incubated for 30 min at room temperature with deprenyl to give a final inhibitor concentration of 10^{-3} to 10^{-10}M. ^{3}H-TMH (20 μl) was then added, followed by 2 h incubation at 37°C. After chromatography, the band corresponding to the cold MIAA marker spot was sectioned, eluted and counted.

(iii) Substrate competition between PEA or 5HT and TMH

A 1% $^{w}/v$ homogenate of human liver in 10 mM phosphate buffer, pH 7.4, was used in this part of the investigation. A mixture comprising 100 μl 100 mM phosphate buffer, pH 7.4, 20 μl of the above homogenate and 20 μl of water or 20 μl 10^{-2} M deprenyl for blanks was prepared. 20 μl of TMH and 20 μl of either ^{14}C-5HT (54 mCi/mmol) or 20 μl of ^{14}C-PEA (48 mCi/mmol) were added whilst the rack of tubes was on

ice. The various concentrations of substrates used are shown in Figures 7:3 and 7:4. The samples were then incubated for 30 min at 37°C, and the reaction stopped by transferring the tubes to an ice-bath and by the addition of 100 µl 2 M citric acid. The products formed were separated by solvent extraction, in the case of PEA by toluene (3 ml) and for 5HT by a 1:1 mixture of toluene and ethyl acetate (3ml). The tubes were then frozen, and whilst in that state the organic phase was poured off and counted, after the addition of Instagel (3 ml).

The effect of deprenyl on PEA oxidation

The method described in Chapter 2 was employed.

Protein concentration

This was measured as described in Chapter 2.

(iv) Counting and extraction efficiencies

Comparative counting efficiencies for ^{3}H (56%) and ^{14}C (97%) were calculated.

The quenching and extraction produced a 41% reduction in the observed TMH counts, whereas only a 5% loss from these sources occurred during PEA and 5HT assay.

Results

Figure 7:2 shows the position of the cold markers for TMH and MIAA and the distribution of radioactivity after ^{3}H-TMH had been incubated with human liver homogenate with or without a MAO-B selective concentration of deprenyl. It will be seen that radioactive peaks correspond with marker spots for both compounds, showing that MIAA was the major metabolite.

Figure 7:3 illustrates the effect of a range of deprenyl concentrations on TMH oxidation in comparison with their effect on the oxidation of PEA by a homogenate of the same liver. Their sensitivity to deprenyl was very similar, as might be predicted if TMH oxidation were similarly carried out by MAO-B and not MAO-A.

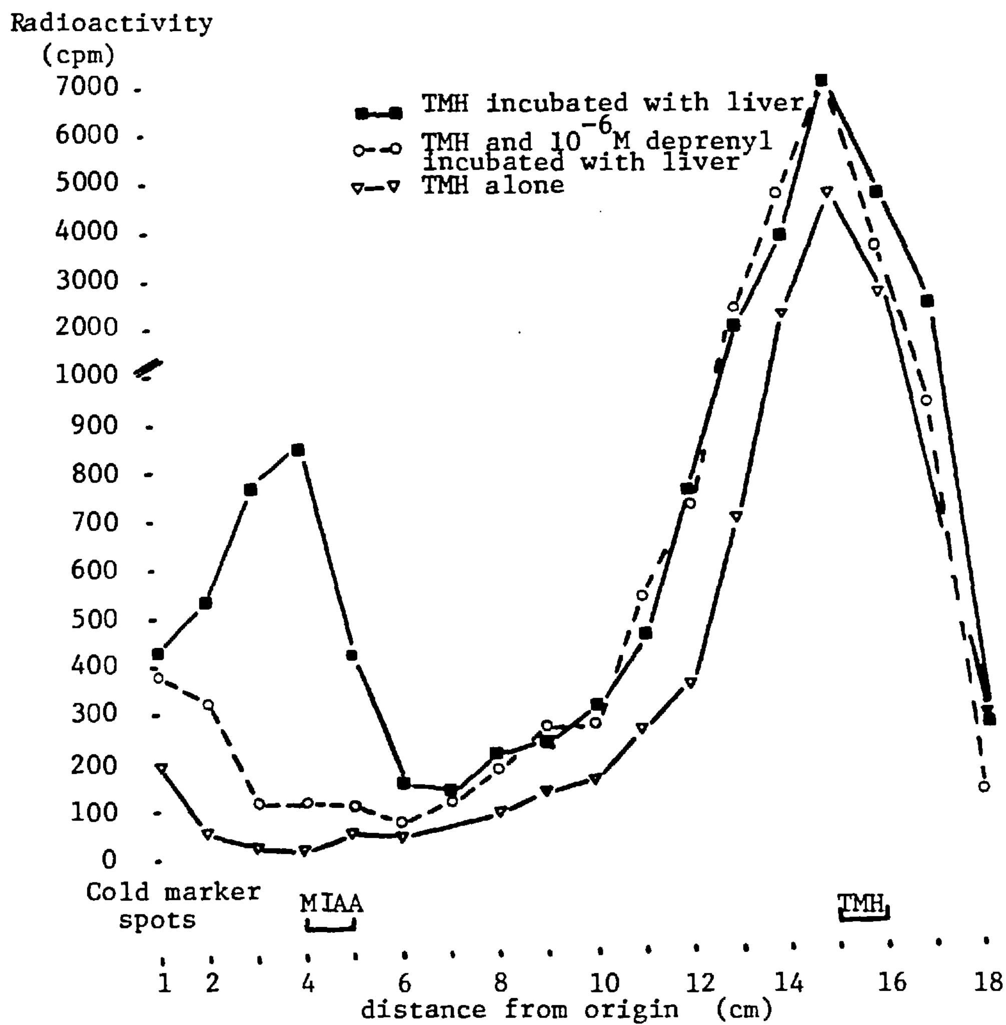


Fig. 7:2 TLC analysis of ^{3}H-TMH metabolism to ^{3}H-MIAA by human liver

The Rf values were 0.22 and 0.88 for MIAA and TMH respectively

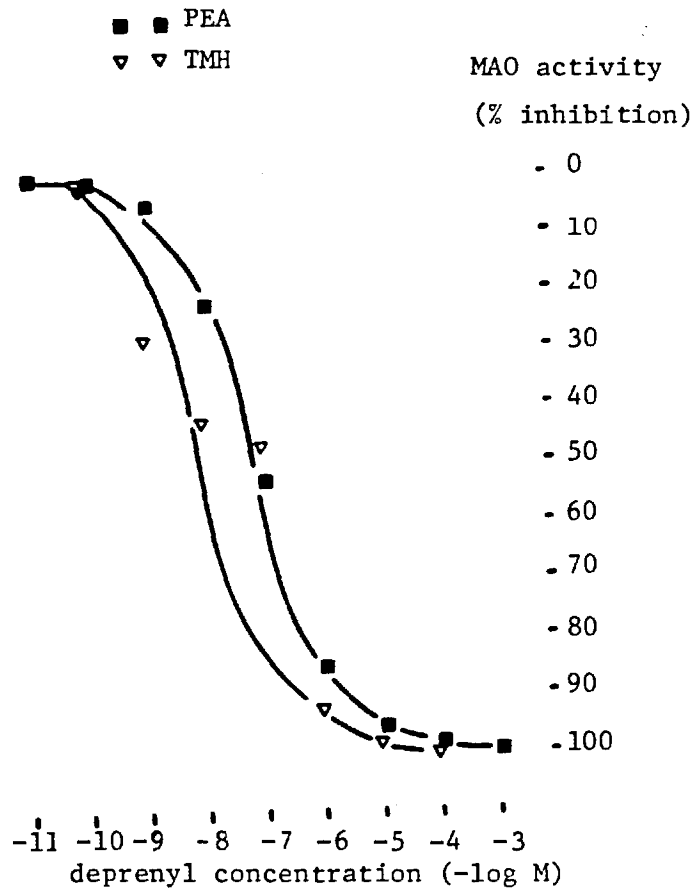


Fig. 7:3 The sensitivity of PEA and TMH oxidation to (-) deprenyl inhibition

The specific activity of human liver for PEA was 17.3 n moles/mg protein/30 min; that for TMH could not be so accurately determined owing to slight uncertainty about purified TMH concentration, but based on the assumption mentioned in the methods section, a value of 1.3 n moles/mg protein/30 min was obtained.

Figures 7:4 and 7:5 show the effect of adding a range of concentrations of unlabelled TMH with ^{14}C-PEA (1-10 μM) or ^{14}C-5HT (100-400 μM). These Lineweaver-Burk plots clearly establish that substrate competition between TMH and PEA occurs. The apparent Ki of TMH with PEA was 127 μM, and the Km of PEA was 6.4 μM. Very much higher concentrations of TMH were required in order to inhibit 5HT oxidation, and even then no clear inhibitory pattern emerged. The Km with 5HT was 100 μM.

Discussion

The results from the deprenyl sensitivity experiments show a similar inhibition profile of TMH and PEA oxidation, inferring that they are metabolized by the same enzyme. 5HT oxidation is about a hundred times more resistant to deprenyl than PEA oxidation (see Chapter 3). In fact, this study is the first demonstration of the *in vitro* conversion of TMH to MIAA in human tissue. The slight difference apparent in the sensitivities of the two amines to MAO inhibition probably stems from the methodology employed: the separation of PEA from its deaminated metabolites is a much easier and more efficient process than is the case with TMH. Indeed the absence of convenient extraction procedures for the histamine metabolites is probably largely responsible for the relative lack of research effort in the area, compared with the catecholamines for example. However, a more elegant method has recently been devised (Hough et al, 1979), which might well initiate a new upsurge of interest into the CNS function of HA.

One other reason for a discrepancy in the deprenyl sensitivity of TMH and PEA might be connected with the specificity of PEA as a MAO-B substrate. After the study was completed it was discovered that contrary to all previous beliefs, PEA at high substrate concentrations is not entirely dependent on MAO-B for deamination. At the concentration

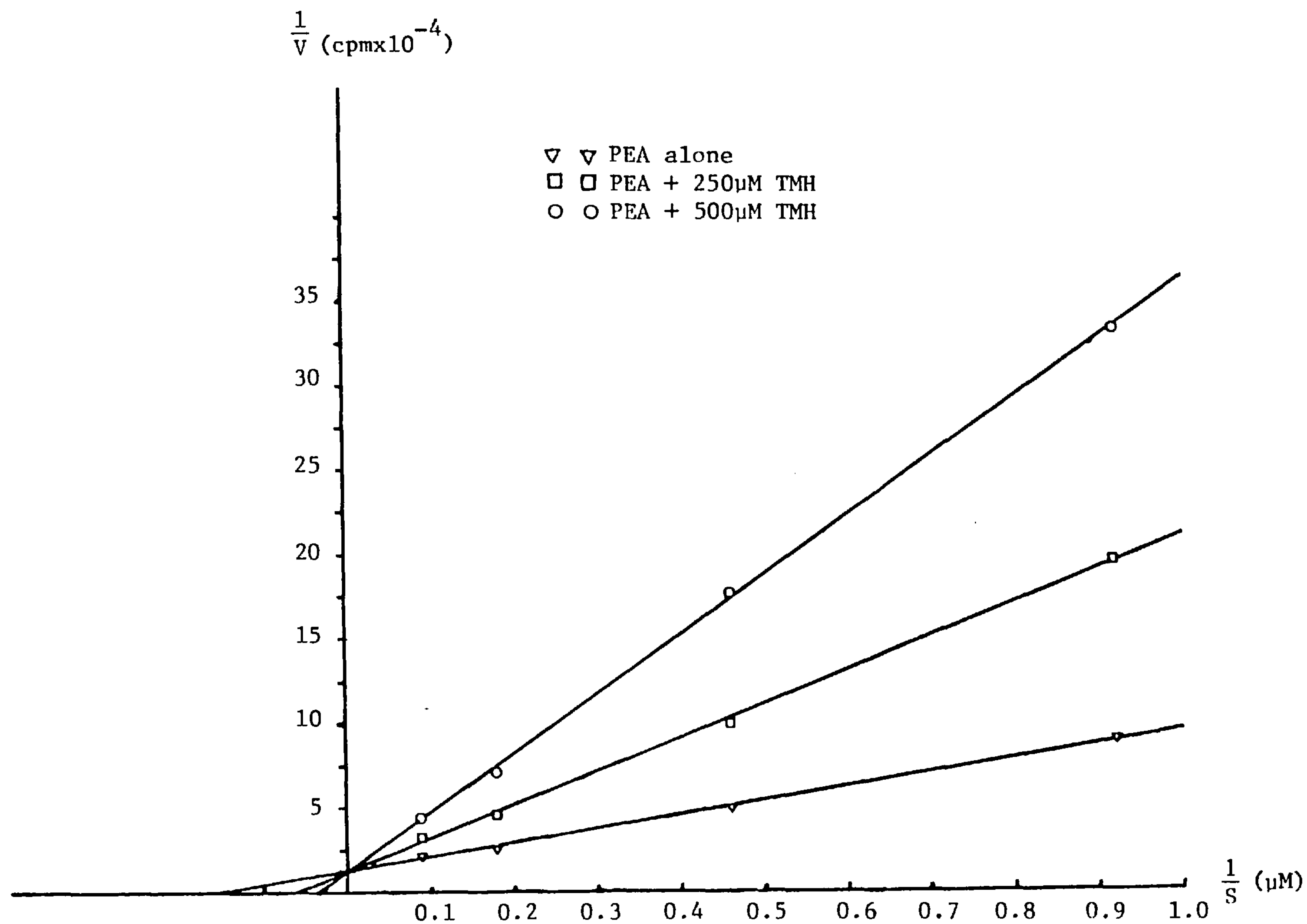


Fig. 7:4 Competitive inhibition of PEA oxidation by TMH

Each point represents the mean of at least two separate experiments performed in duplicate.

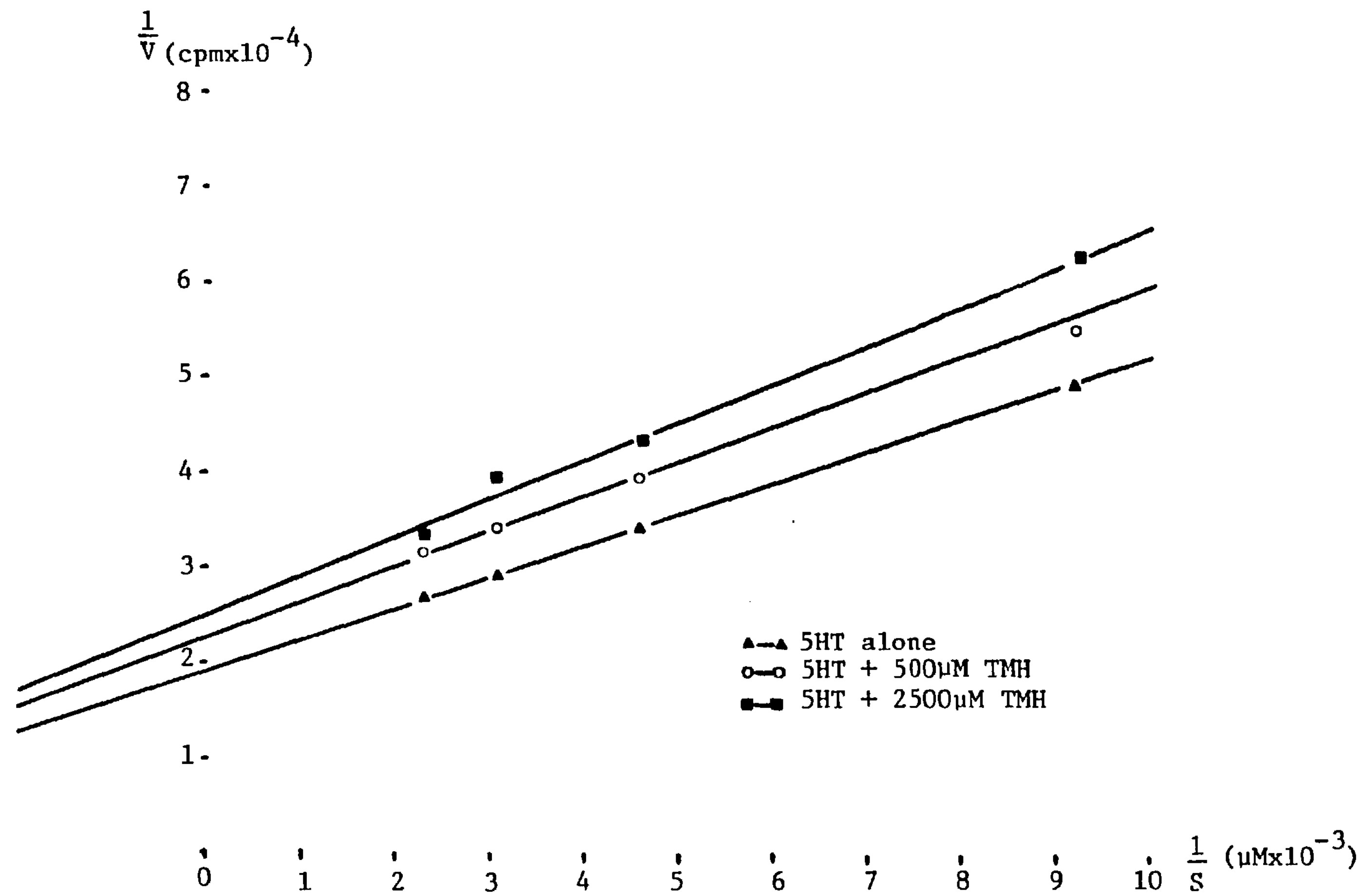


Fig. 7:5 Effect of TMH on 5HT oxidation

Each point represents the mean of two separate determinations, each done in duplicate.

used in this part of the study, 125 μM and above, it tends also to be metabolized by MAO-A (Suzuki et al, 1979) although with a higher Km than with MAO-B (Lewinson et al, 1980).

The substrate competition experiments show clearly that TMH is a substrate for MAO-B in human liver and not a related deprenyl-sensitive enzyme. Even small concentrations of TMH markedly reduced the rate of PEA oxidation by MAO, whilst leaving Vmax unchanged. In contrast, high concentrations of TMH were needed to affect the rate of oxidation of the MAO-A substrate, 5HT, by MAO. Even when this was achieved, the mode of inhibition certainly was not competitive.

The specific activity of the oxidation of TMH, 1.3 n moles/mg protein/30 min found for human liver, was not too dissimilar from the value for rat liver of 3.6 n moles/mg protein/30 min (Suzuki et al, 1979). PEA was found to be oxidised at about fifteen times the rate of TMH in human liver.

Hough and Domino (1979b) found an extremely high Km value, 3.7 mM, for TMH in rat brain, indicating a low affinity of the substrate for the enzyme. This is contrary to the present findings using human liver, where, although a direct Km was not obtained, the Ki value was 127 μM. In the situation of simple competition between two substrates for the same enzyme form, the Ki value should equal the Km value. Suzuki et al (1979) reported a figure of 38 μM for TMH in rat liver. The Km values obtained here for 5HT and PEA are very like those reported by White and Wu (1975) who also used human tissue, and the value for TMH found in the present study is typical of substrates for MAO.

The competition between substrates, used _in vitro_ to demonstrate the affinity for a common enzyme active site, could also occur _in vivo_. Thus if two MAO-B substrates co-exist in a compartment where MAO is present, they will each tend to prevent the destruction of the other. The substrates with the low Km values (e.g. PEA) have greatest affinity for the enzyme, so such compounds will be the most effective in raising the concentration of other substrates. The finding

that TMH is also a type B substrate raises the possibility that it too could affect the catabolism of other MAO-B substrates (e.g. DA in the brain). This interaction would presumably be pronounced after MAO inhibition.

The function of MAO-B has always been puzzling. It has a distribution quite distinct from that of MAO-A (see Chapter 5), and yet, until this study, the only known endogenous substrate in man relying entirely on MAO-B for metabolism was PEA. The discovery of a new amine which does likewise may help to rationalise the existence of MAO-B. Any role of PEA in brain function is far from certain, although it has been implicated in the affective group of disorders (Sabelli et al, 1978a). Interest in the central actions of HA has been stimulated by the finding that clinically effective tricyclic antidepressants block HA H_2 receptors in guinea-pig brain (Green and Maayani, 1977), with a potency at least comparable with their ability to inhibit the uptake of 5HT and NA, the previously assumed site of action of this group of drugs. Another study (Tran et al, 1978) showed that tricyclics are even more potent at inhibiting H_1 receptors in rat and calf brain. In fact, of those drugs tested, they were the most powerful in this respect. Moreover, iprindole, a tricyclic antidepressant which does not affect 5HT and NA uptake, is an effective antagonist of H_1 and H_2 receptors.

The MAO inhibitors can also be effective antidepressants (Robinson et al, 1973), their assumed mode of action being to increase the synaptic concentration of MAO substrates deficient in depression. The controversy over the amines actually involved was recently investigated in a clinical trial of the value of the selective MAO-B inhibitor, pargyline, as opposed to the selective MAO-A inhibitor, clorgyline (Lipper et al, 1979). In the outcome, clorgyline was found to have greater antidepressant properties, making the hypothesis that MAO-B substrates are solely involved in depression unlikely, and implying significant roles for 5HT and NA. Whether the antidepressant effect of MAO inhibitory drugs derives from an interaction with HA systems seems doubtful; in fact, Kanof

and Greengard (1978) found drugs of this class did not alter histamine-stimulated adenylate cyclase.

Although the clinical use of deprenyl seems to be associated with relatively few side effects, in the light of the results presented here, adverse reactions stemming from an interference with HA metabolism should be borne in mind.

CHAPTER EIGHT - GENERAL DISCUSSION

Although the synthesis of selective MAO inhibitors is certainly a great advance in MAO research, their arrival has not simplified the field of study; rather, it has brought in its wake fresh problems.

Deprenyl, the first selective MAO-B inhibitor to be used clinically, is the subject of much of the work presented here. Using 5HT and PEA, respectively, as specific A and B substrates, the selective inhibitory effect of deprenyl was demonstrated in a number of human tissues (Chapter 3 and 5). Morever, a potent and rapid inhibition of platelet MAO activity in vivo was noted; and of the endogenous urinary substrates monitored, it appeared that PEA excretion best reflected overall body MAO-B inhibition (Chapter 3).

Clinical studies confirmed that deprenyl is a useful adjuvant to L-dopa therapy in certain cases of Parkinson's disease, and it was shown that platelet MAO activity is equally susceptible to inhibition in these patients as in normal controls (Chapter 4). However, unlike normal subjects, parkinsonians did not consistently excrete a raised concentration of PEA during deprenyl administration; although, subsequent investigations revealed that the subjects under scrutiny were also receiving a peripheral decarboxylase inhibitor and that this type of drug prevents the increase in output of PEA caused by deprenyl, indicating the importance of AAAD in the biosynthesis of PEA (Chapter 4).

Despite the great selectivity of deprenyl as a MAO inhibitor, it is by no means a "pure" drug. Indeed, the drug was found to be broken down in vivo to methamphetamine and amphetamine (Chapter 6). However, in parkinsonian patients, by manipulating the excretion rate of these compounds, and by administering them directly as a substitute for deprenyl, it was possible to deduce that its therapeutic effect does not rely on pharmacologically-active metabolites (Chapter 6).

Studies on post-mortem human tissue showed that there is an uneven distribution of MAO-A and MAO-B activities in the periphery and even within discrete brain regions (Chapter 5). DA oxidation was found to be particularly susceptible to MAO-B inhibition in striatal areas and as this region is depleted of DA in Parkinson's disease, the improvement derived by some parkinsonians can be explained in terms of MAO inhibition by deprenyl (Chapter 5).

However, perhaps the most important feature of deprenyl is probably not due to its MAO inhibitory properties alone. The absence of the "cheese effect" in normals and parkinsonians is, so far, a unique attribute of an irreversible MAO inhibitor (Chapter 3). It was originally considered that the safety of deprenyl results from its selective action on MAO-B. The MAO gut barrier is of the A variety (Chapter 5), which might conceivably remain intact during treatment with suitable doses of a selective MAO-B inhibitor, thus preventing harmful quantities of TYR entering the circulation. However, it now seems that the "cheese effect" is unlikely to result from MAO inhibition alone, but that some additional pharmacological effect on the NA neurones is necessary. It seems increasingly likely that deprenyl, by not possessing this latter property (Knoll and Magyar, 1972; Sandler et al, 1978), escapes the TYR-potentiating effect of other MAO inhibitors.

Thus, even though the abscence of the "cheese effect" with deprenyl is not a transient phenomena (Chapter 3), this property is not necessarily an indication that the drug remains selective. Indeed, it is clear that deprenyl is not as selective _in vivo_ as _in vitro_ (Riederer et al., 1978), but the precise effect on MAO-A and its substrates remains unknown and this is one problem which requires urgent attention. It has remained unanswered so far because of the absence of satisfactory MAO-A counterparts to the platelet, a readily accessible tissue with MAO-B activity and to urinary PEA, a compound the concentration of which is an index of overall body MAO-B inhibition (Chapter 3). One solution to this problem would be to perform experiments in a species shown to have similar MAO substrate specificities and MAO-A/B distribution. Recent studies in the vervet monkey indicate that this animal may be a suitable model (Murphy et al., 1979).

Another outstanding question concerns the exact mechanism of the "cheese effect." One way to assess the importance of the MAO-A gut barrier would be to measure plasma TYR concentration at intervals following the ingestion of TYR in subjects receiving either deprenyl, a conventional MAO inhibitor or no other treatment. In addition, in a suitable animal model, it would be interesting to examine the extent to which different MAO inhibitors affect the noradrenergic neurones, and whether in fact this relates to their ability to to produce the "cheese effect."

Even though the precise nature of the multiple forms of MAO remains unresolved, it seems likely that the real situation is more complex than the simple division into A and B forms. The function of the B variety is somewhat obscure, but the discovery that TMH relies on MAO-B for its metabolism (Chapter 7) indicates that at least one of the roles of the enzyme may be to control HA concentration. The effect of deprenyl on PEA (Chapter 3) highlights the importance which the so-called trace amines may have when MAO activity is suppressed, and subsequent research may well reveal other substrates which depend on MAO-B for oxidation.

Our clinical work with deprenyl showed that a MAO inhibitor can be used safely and effectively in the treatment of Parkinson's disease (Chapters 3 and 4). Other disorders have previously been found to benefit from the administration of MAO inhibitors, but their use is restricted because of the "cheese effect." These included depression (Robinson et al, 1973), migraine (Anthony and Lance, 1969), phobia (Kelly et al, 1970), and narcolepsy (Wyatt et al., 1971a). In the light of the recent finding that deprenyl shares the ability of conventional inhibitors to reduce REM sleep (Thornton, Dore, Elsworth, Herbert, Stern-sumitted for publication), it is worth noting that the mood changes in depressed subjects following phenelzine treatment parallels the time course of REM sleep suppression (Dunleavy and Oswald, 1973; Wyatt et al., 1971b) and that the narcolepsy syndrome resembles, in certain ways, REM sleep itself (Wyatt et al., 1971a).

If deprenyl proved to be unsuccessful in treating such conditions, it would be interesting to investigate whether the co-administration of deprenyl and a conventional MAO inhibitor is an effective, yet safe, remedy.

The safety, clinical potency and selectivity of inhibition indicates that deprenyl may become an important drug, not only as a basic research tool, but also in clinical practice. Certainly, it provides impetus to the development of new selective or unselective MAO inhibitors (e.g., Knoll et al., 1978) which may well have a promising future.

REFERENCES

Abou, Y.Z., Adam, H.M., Stephen, W.R.G. (1973) Brit. J. Pharmacol. 48, 577.

Achee, F.M., Gabay, S., Tipton, K.F. (1977) Prog. Neurobiol. 8, 325.

Alles, G.A., Heegard, E.V. (1943) J. Biol. Chem. 147, 487.

Andén, N. E., Carlsson, A., Dahlstrom, A., Fuxe, K., Hillarp, N.-A., Larsson, K. (1964) Life Sci. 3, 523.

Antelman, S.M., Edwards, D.J., Lin, M. (1977) Brain Res. 127,317.

Anthony, M., Lance, J.W. (1969) Arch. Neurol. 21, 263.

Asatoor, A.M., Levi, A.J., Milne, M.D. (1963) Lancet ii, 733.

Bachur, N.R., Udenfriend, S. (1966) J. Biol. Chem. 241, 1308.

Baker, G.B., Raiteri, M., Bertollini, R., del Carmine, P.E., Keane, P.E., Martin, I.L. (1976) J. Pharm. Pharmacol. 28, 456.

Ball, S.G., Lee, M.R. (1977) Brit. J. Clin. Pharmacol. 4, 115.

Ball, S.G., Oates, N.S., Lee, M.R. (1978) Clin. Sci. Mol. Med. 54, 29 P.

Barbeau, A. (1961) Proc. 7th Int. Congr. Neurol., Rome. Vol. 2 p 925 Societa Grafica Romana, Rome.

Barbeau, A. (1962) Canad. Med. Ass. J. 87, 802.

Barbeau, A., McDowell, F.H. (1970) eds. L-Dopa and Parkinsonism. Davis, Philadelphia.

Barbeau, A., Sourkes, T.L., Murphy, G.F. (1962) In: Monoamines et Système Nerveux Central (ed. De Ajuriaguerra, J.) p 247 Georg, Genève.

Baudry, M., Martres, M.P., Schwartz, J.C. (1973a) J. Neurochem. 21, 1301.

Baudry, M., Chast, F., Schwartz, J.C. (1973b) J. Neurochem. 20, 13.

Beckett, A.H., Rowland, M. (1965a) Nature 206, 1260.

Beckett, A.H., Rowland, M. (1965b) J. Pharm. Pharmacol. 17, 628.

Beckett, A.H., Rowland, M. (1965c) J. Pharm. Pharmacol. 17, 109 S.

Behringer, K., Wilmanns, K. (1929) Dtsch. Med. Wschr. 55, 2081.

Bennett, C.T., Pert, A. (1974) Brain Res. 78, 151.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O. (1961) Klin. Wschr. 39, 1056.

Bernheimer, H. Birkmayer, W., Hornykiewicz, O. (1963) Klin. Wschr. 41,465.

Bethune, H.C., Burrell, R.H., Culpan, R.H., Ogg, G.J. (1964) Amer. J. Psychiat. 121,245.

Bianchine, J.R., Messiha, F.S., Preziosi, T.J. (1973) Adv. Neurol. (ed. Yahr, M.D.) Vol. 2 p 101 Raven, New York.

Birkmayer, W. (1966) Wien Z. Nervenheilk. 23,128.

Birkmayer, W. (1969) Wien. Klin. Wschr. 81,677.

Birkmayer, W. (1976) In: Advances in Parkinsonism (ed. Birkmayer, W., Hornykiewicz, O.) p 407 Roche, Basel.

Birkmayer, W. (1978) J. Neural Transmiss. 43,239.

Birkmayer, W., Hornykiewicz, O. (1961) Wien. Klin. Wschr. 73,787.

Birkmayer, W., Hornykiewicz, O. (1962) Arch. Psychiat. Nervenkr. 203,560.

Birkmayer, W., Riederer, P., Ambrozi, L., Youdim, M.B.H. (1977) Lancet i,439.

Birkmayer, W., Riederer, P. Youdim, M.B.H., Linauer, W. (1975) J. Neural Transmiss. 36,303.

Black, J.W., Duncan, W.A.M., Durant, C.J., Ganellin, C.R., Parsons, M.E. (1972) Nature 236,385.

Black, J.W., Ganellin, C.R. (1974) Experientia 30,111.

Blackwell, B. (1963a) Lancet i,849

Blackwell, B. (1963b) Lancet i,167.

Blackwell, B. (1963c) Lancet ii,614.

Blackwell, B., Mabbitt, L.A. (1965) Lancet i,938.

Blackwell, B. Marley, E. (1966) Brit. J. Pharmacol. 26,120.

Blackwell, B., Marley, E., Mabbitt, L.A. (1965) Lancet i,940.

Blackwell, B., Marley, E., Price, J., Taylor, D. (1967) Brit. J. Psychiat. 113,349.

Blaschko, H. (1952) Pharmacol. Rev. 4,415.

Blaschko, H. (1957) Experientia 13,9.

Blaschko, H. (1966) In: Molecular Basis of Some Aspects of Mental Activity (ed. Walaas, O.) Vol 1 p 403 Academic Press, London.

Blaschko, H. Richter, D., Schlossman, H. (1937) Biochem. J. 31,2187.

Blau, K., Claxton, I.M., Ismahan, G., Sandler, M. (1979) J. Chromatog. 163, 135.

Boulton, A.A., Dyck, L.E., Durden, D.A. (1974) Life Sci. 15,1673.

Braestrup, C., Andersen, H., Randrup, A. (1975) Eur. J. Pharmacol. 34,181.

Brodie, B.B., Hogben, C.A.M. (1957) J. Pharm. Pharmacol. 9,345.

Brownstein, M.J., Saavedra, J.M., Palkovits, M.,Axelrod, J., (1974) Brain Res. 77, 151.

Burkhard, W.P., Gey, K.F., Pletscher, A. (1963) J. Neurochem. 10,183.

Burn, J.H., Rand, M.J. (1958) J. Physiol. 144,314.

Calcutt, C.R. (1976) Gen. Pharmacol. 7,15.

Caldwell, J. (1976) Drug Metab. Rev. 5,219.

Caldwell, J., Dring, L.G., Williams, R.T. (1972) Biochem. J. 129,11.

Callingham, B.A., Lyles, G.A. (1975) Brit. J. Pharmacol. 53,458 P.

Cannon, P.J. (1977) Science 196,1322.

Carlsson, A. (1959) Pharmacol. Rev. 11,490.

Carlsson, A., Linqvist, M., Magnusson, F., Waldeck, B. (1958) Science 125,471.

Cavanaugh, J.H., Griffith, J.D., Oates, J.A. (1970) Clin. Pharmacol. Ther. 11,656.

Challacombe, D.N., Sandler, M., Southgate, J. (1971) Arch. Dis. Childh. 46,213.

Chen, K.K. (1927) Arch. Int. Med. 39,404.

Chesrow, E.J., Musci, J.P., Kaplitz, S.E., Sabatini, R., Levine, J.M. (1962) Geriatrics 17,137.

Christensen, N.J. (1973) Scand. J. Clin. Lab. Invest. 31,343.

Cooper, A.J., Magnus, R.V., Rose, M.J. (1964) Lancet i,527.

Cooper, H.A., Gunn, J.A. (1931) Lancet ii,901.

Cotzias, G.C., Van Woert, M.H., Schiffer, L.M. (1967) New Engl. J. Med. 276,374.

Coyle, J.T., Snyder, S.H. (1969a) Science 166,899.

Coyle, J.T., Snyder, S.H. (1969b) J. Pharmacol. Exp. Ther. 170,221.

Csanda, E., Antal, J., Antóny, M., Csanaky, A. (1978) J. Neural Transmiss. 43,263.

Dale, H.H., Dixon, W.E. (1909) J. Physiol. 39,25.

Davidson, J., McLeod, M.N., White, H.L. (1978) Amer. J. Psychiat. 135, 470.

Davison, A.N. (1958) Physiol. Rev. 38, 729.

DeArmond, S.J., Fusco, M.M., Dewey, M.M. (1976) Structure of the Human Brain. Oxford University Press, New York.

De Villiers, J.C. (1966) Brit. J. Psychiat. 112, 109.

Dixon, M., Webb, E.C. (1958) Enzymes p91 Academic Press, New York.

Donnelly, C.H., Murphy, D.L. (1977) Biochem. Pharmacol. 26, 853.

Douglas, W.W. (1975) In: The Pharmacological Basis of Therapeutics. (ed. Goodman, L.S., Gilman, A.) p590. Macmillan, New York.

Dunleavy, D.L.F., Oswald, I. (1973) Arch. Gen. Psychiat. 28,353.

Durden, D.A., Philips, S.R., Boulton, A.A. (1976) Biochem. Pharmacol. 25, 858.

Dyck, L.E., Boulton, A.A. (1975) Res. Comm. Chem. Pathol. Pharmacol. 11,73.

Edwards, D.J. (1978) Life Sci. 23,1201.

Edwards, D.J., Blau, K. (1973) Biochem. J. 132,95.

Edwards, D.J., Chang, S.S. (1975) Biochem. Biophys. Res. Comm. 65,1018.

Edwards, D.J., Malsbury, C.W. (1977) Life Sci. 21,1009.

Egashira, T. (1976) Jap. J. Pharmacol. 26,493.

Egashira, T., Ekstedt, B., Oreland, L. (1976) Biochem. Pharmacol. 25,2583.

Ehringer, H., Hornykiewicz, O. (1960) Klin. Wschr. 38,1236.

Ekstedt, B., Oreland, L. (1976) Arch. Int. Pharmacodyn. 222,157.

Evans, D.A.P., Davison, K., Pratt, R.T.C. (1965) Clin. Pharmacol. Ther. 6,430.

Fellows, L.E., King, G.S., Pettit, B.R., Goodwin, B.L., Ruthven, C.R.J., Sandler, M. (1978) Biomed. Mass. Spectrom. 5,508.

Findlay, L. (1911) Quart. J. Med. 4,489.

Fischer, A.G., Schultz, A.R., Oliner, L. (1966) Life Sci. 5,995.

Fowler, C.J., Callingham, B.A., Mantle, T.J., Tipton, K.F. (1978) Biochem. Pharmacol. 27,97.

Fram, D.H., Green, J.P. (1968) Clin. Pharmacol. Ther. 9,355.

Fuller, R.W. (1972) In: Monoamine Oxidase - New Vistas (ed. Costa, E., Sandler, M.) Adv. Biochem. Psychopharmacol. Vol. 5 p 339 Raven, New York.

Garbarg, M., Barbin, G. (1976) Brain Res. 106,333.

Garbarg, M., Baudry, M., Benda, P., Schwartz, J.C. (1975) Brain Res. 83,538.

Garland, H., Pearce, J. (1967) Quart. J. Med. 36, 445.

Gerald, M.C., Maickel, R.P. (1972) Brit. J. Pharmacol. 44,462.

Glover, V., Sandler, M., Owen, F., Riley, G.J. (1977) Nature 265,80.

Goodwin, B.L. (1976) Handbook of Intermediary Metabolism of Aromatic Compounds. Chapman and Hall, London.

Goodwin, B.L., Ruthven, C.R.J., Fellows, L.E., Sandler, M. (1976) Clin. Chim. Acta 73,191.

Gorkin, V.Z., Orekhovitch, W.N. (1967) Biochim. Appl. 14,343.

Gottfries, C.G., Magnusson, T. (1962) Acta Psychiat. Scand. 38,223.

Gottfries, C.G., Oreland, L., Wiberg, A., Winblad, B. (1975) J. Neurochem. 25,667.

Green, A.R., Mitchell, B.D., Tordoff, A.F.C., Youdim, M.B.H. (1977) Brit. J. Pharmacol. 60,343.

Green, J.P. (1964) Fed. Proc. 23,1095.

Green, J.P. (1970) In: Handbook of Neurochemistry. (ed. Lajtha, A.) Vol 4 p221. Plenum, New York.

Green, J.P., Johnston, C.L., Weinstein, H. (1978) In: Psychopharmacology - a Generation of Progress. (ed. Lipton, M., DiMascio, A., Killam, K.) p319. Raven, New York.

Green, J.P., Maayani, S. (1977) Nature 269,163.

Grote, S.S., Moses, S.G., Robins, E., Hudgens, R.W., Croninger, A.B. (1974) J. Neurochem. 23,791.

Guggenheim, M. (1913) Z. Physiol. Chem. 88,276.

Haas, H.L. (1974) Brain Res. 76,363.

Haas, H.L., Anderson, E.G., Hösli, L. (1973) Brain Res. 51,269.

Hardegg, W., Heilbronn, E. (1961) Biochim. Biophys. Acta 51,553.

Hare, M.L.C. (1928) Biochem. J. 22,968.

Harley, V. (1913) Proc. Roy. Soc. Med. 6,1.

Harris, J.E., Baldessarini, R.J. (1973) Neuropharmacology 12,669.

Hayaishi, O., Tabor, H., Hayaishi, T. (1954) J. Amer. Chem. Soc. 76,5570.

Hedberg, D.L., Gordon, M.W., Glueck, B.C. (1966) Amer. J. Psychiat. 122,933.

Heller, B. (1978) In: Noncatecholic Phenylethylamines (ed. Mosnaim, A.D., Wolf, M.E.) Part I p397 Dekker, New York.

Heller, B., Fischer, E. (1973) Arzneimittel-forschung 23,884.

Hendley, E.D., Snyder, S.H. (1968) Nature 220,1330.

Hirano, M., Uchimura, H., Saito, M. (1975) Brain Res. 93,558.

Hodge, J.V., Nye, E.R., Emerson, G.W. (1964) Lancet i,1108.

Hoeldtke, R.D., Wurtman, R.J. (1974) Metabolism 23,33.

Holmes, J.C., Rutledge, C.O. (1976) Biochem. Pharmacol. 25,447.

Holzer, G., Hornykiewicz, O. (1959) Arch. Exp. Pathol. Pharmakol. 237,27.

Horn, A.S. (1973) Brit. J. Pharmacol. 47,332.

Hornykiewicz, O., (1973) In: Adv. Neurol. (ed. Yahr, M.D.) Vol 2 p1 Raven, New York.

Hornykiewicz, O., Lloyd, K.G., Davidson, L. (1976) In: GABA in Nervous System Function (ed. Roberts, E., Chase, T.N., Tower, D.B.) p479 Raven, New York.

Horwitz, D., Lovenberg, W., Engelman, K., Sjoerdsma, A. (1964) J. Amer. Med. Ass. 188,1108.

Hough, L.B., Domino, E.F. (1979a) J. Neurochem. 32,1865.

Hough, L.B., Domino, E.F. (1979b) J. Pharmacol. Exp. Ther. 208,442.

Hough, L.B., Stetson, P.L., Domino, E.F. (1979) Anal. Biochem. 96,56.

Houslay, M.D. (1977) J. Pharm. Pharmacol. 29,664.

Houslay, M.D., Garrett, N.J., Tipton, K.F. (1974) Biochem. Pharmacol. 23,1937.

Houslay, M.D., Tipton, K.F. (1973) Biochem. J. 135,173.

Houslay, M.D., Tipton, K.F. (1974) Biochem. J. 139,645.

Hsu, L.L., Mandell, A.J. (1973) Life Sci. 13,847.

Hunter, K.R., Boakes, A.J., Laurence, D.R., Stern, G.M. (1970) Brit. Med. J. 3,388.

Innes, I.R., Nickerson, M. (1975) In: The Pharmacological Basis of Therapeutics (ed. Goodman, L.S., Gilman, A.) p477 Macmillan, New York.

Iversen, L.L. (1973) Brit. Med. Bull. 29,130.

Jackson, D.M. (1978) In: Noncatecholic Phenylethylamines (ed. Mosnaim, A.D., Wolf, M.E.) Part I p289 Dekker, New York.

Johnston, J.P. (1968) Biochem. Pharmacol. 17,1285.

Jouvet, M. (1969) Science 163,32.

Kakiuchi, S., Rall, T.W. (1968) Mol. Pharmacol. 4,367.

Kanof, P.D., Greengard, P. (1978) Nature 272,329.

Kapeller-Adler, R., Iggo, B. (1957) Biochim. Biophys. Acta 25,394.

Karjala, S.A., Turnquest, B.W. (1955) J. Amer. Chem. Soc. 77,6358.

Kataoka, K., De Robertis, E. (1967) J. Pharmacol. Exp. Ther. 156,114.

Kebabian, J.W., Calne, D.B. (1979) Nature 277,93.

Kelly, D., Guirguis, W., Frommer, E., Mitchell-Heggs, N., Sargant, W. (1970) Brit. J. Psychiat. 116,387.

Kim, H.C., D'Iorio, A. (1968) Canad. J. Biochem. 46,295.

Kim, J.S., Hirano, M., Uchimura, H., Saito, M., Matsumoto, T., Nakahara, T. (1979) J. Neurochem. 32,253.

Klawans, H.L., Margolin, D.I. (1975) Arch. Gen. Psychiat. 32,725.

Klingman, G.I. (1966) Biochem. Pharmacol. 15,1729.

Knight, R.I., Smith, I.R. (1979) Brit. J. Pharmacol. 64,404P.

Knoll, J. (1976) In: Monoamine Oxidase and Its Inhibition (ed. Wolstenholme, G.E.W., Knight, J.) Ciba Foundation Symposium 39 p135 Elsevier, Amsterdam.

Knoll, J. (1978) J. Neural Transmiss. 43,177.

Knoll, J. Ecsery, Z., Kelemen, K., Nievel, J., Knoll, B. (1965) Arch. Int. Pharmacodyn. 155,154.

Knoll, J., Ecsery, Z., Magyar, K., Sátory, E. (1978) Biochem. Pharmacol. 27,1739.

Knoll, J., Magyar, K. (1972) In: Monoamine Oxidase - New Vistas (ed. Costa, E., Sandler, M.) Adv. Biochem. Psychopharmacol. Vol 5 p393 Raven, New York.

Knoll, J., Vizi, E.S., Somogyi, G. (1968) Arzneimittel-forschung 18,109.

Krasner, N., Cornelius, J.M. (1970) Brit. Med. J. 4,496.

Krayenbühl, H., Siegfried, J. (1970) Neuro-chirugie 16,71.

Krikler, D.M., Lewis, B. (1965) Lancet i,1166.

Lader, M.H., Sakalis, G., Tansella, M. (1970) Psychopharmacologia 18,118.

Langer, S.Z. (1970) J. Physiol. 208,515.

Lee, K.Y., Beilin, L.J., Vandongen, R. (1979) Lancet i,1110.

Levine, R.J., Sjoerdsma, A. (1963) Clin. Pharmacol. Ther. 4,22.

Lewinsohn, R., Glover, V., Sandler, M. (1980) Biochem. Pharmacol. 29,777.

Lipper, S., Murphy, D.L., Slater, S., Buchsbaum, M.S. (1979) Psychopharmacology 62,123.

Lloyd, K.G., Davidson, L., Hornykiewicz, O. (1975) J. Pharmacol. Exp. Ther. 195,453.

Long, R.F. (1962) Acta Neurol. Scand. 38, S1, 27.

Loo, Y.H. (1967) J. Neurochem. 14,813.

Loomer, H.P., Saunders, J.C., Kline, N.S. (1957) Amer. Psychiat. Ass. Psychiat. Res. Rep. 8,129.

Lovenberg, W., Weissbach, H., Udenfriend, S. (1962) J. Biol. Chem. 237,89.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951) J. Biol. Chem. 193,265.

Mackay, A.V.P., Davies, P., Dewar, A.J., Yates, C.M. (1978) J. Neurochem. 30,827.

Magyar, K., Visi, E.S., Ecsery, Z., Knoll, J. (1967) Acta Physiol. Acad. Sci. Hung. 32,377.

Mantegazza, P., Riva, M.J. (1963) J. Pharm. Pharmacol. 15,472.

Marks, J. (1965) In: The Scientific Basis of Drug Therapy in Psychiatry. (ed. Marks, J., Pare, C.M.B.) p191 Macmillan, New York.

Marley, E., Blackwell, B. (1970) Adv. Pharmacol. Chemother. 8,185.

Marsden, C.D., Parkes, J.D. (1976) Lancet i,292.

Marshall, E.F. (1976) Brit. Med. J. 2,817.

Maśliński, Cz., Lebrecht, U., Nowak, J.Z., Pilc, A. (1973) Agents and Actions 3,185.

Matin, S.B., Rowland, M. (1972) J. Pharm. Sci. 61,1235.

Maycock, A.L., Abeles, R.H., Salach, J.I., Singer, T.P. (1976) In: Monoamine Oxidase and Its Inhibition (ed. Wolstenholme, G.E.W., Knight, J.) Ciba Foundation Symposium 39 p33 Elsevier, Amsterdam.

McGeer, P.L., Boulding, J.E., Gibson, W.C., Foulkes, R.G. (1961) J. Amer. Med. Ass. 177.665.

Medical Research Council (1965) Brit. Med. J. 1,881.

Mena, I., Marin, O., Fuenzalida, S., Cotzias, G.C. (1967) Neurology 17, 128.

Miller, H.H., Clarke, D.E. (1978) Comm. Psychopharmacol. 2,319.

Murphy, D.L., (1978) Biochem. Pharmacol. 27,1889.

Murphy, D.L., Brand, E., Goldman, T., Baker, M., Wright, C., Van Kammen, D., Gordon, E. (1977) J. Nerv. Ment. Dis. 164,129.

Murphy, D.L., Costa, J.L., Shafer, B., Corash, L. (1978) Psychopharmacology 59,193.

Murphy, D.L., Redmond, D.E., Garrick, N., Baulu, J. (1979) Neurochem. Res. 4,53.

Nakajima, T., Sano, I. (1964) Biochim. Biophys. Acta 82,260.

Nuessle, W.F., Norman, F.C., Mille, H.E. (1965) J. Amer. Med. Ass. 192,726.

Oates, J.A., Nirenberg, P.Z., Jepson, J.B., Sjoerdsma, A., Udenfriend, S. (1963) Proc. Soc. Exp. Biol. Med. 112,1078.

Ordenstein, L. (1867) M.D. Thesis. Martinet, Paris.

Oreland, L. (1972) In: Monoamine Oxidase - New Vistas (ed. Costa, E., Sandler, M.) Adv. Biochem. Psychopharmacol. Vol 5 p37 Raven, New York.

Owen, F., Cross, A.J., Lofthouse, R., Glover, V. (1979) Biochem. Pharmacol. 28,1077.

Pakkenberg, H., Brody, H. (1965) Acta Neuropathol. 5,320.

Pare, C.M.B. (1976) In: Monoamine Oxidase and its Inhibition (ed. Wolstenholme, G.E.W., Knight, J.) Ciba Foundation Symposium 39 p271 Elsevier, Amsterdam.

Parkes, J.D., Tarsy, D., Marsden, C.D., Bovill, K.T., Phipps, J.A., Rose, P., Asselman, P. (1975) J. Neurol. Neurosurg. Psychiat. 38,232.

Parkinson, J. (1817) An Essay on the Shaking Palsy. Sherwood, Neely, Jones, London.

Pearce, I., Pearce, J.M.S. (1978) Brit. Med. J. 1,1402.

Phillis, J.W., Tebēcis, A.K., York, D.B. (1968) Brit. J. Pharmacol. 33,426.

Pisano, J.J. (1960) Clin. Chim. Acta 5,406.

Poirier, L.J., Sourkes, T.L. (1965) Brain 88,181.

Pollard, H., Bischoff, S., Schwartz, J.C. (1973) Eur. J. Pharmacol. 24,399.

Pollard, H., Bischoff, S., Schwartz, J.C. (1974) J. Pharmacol. Exp. Ther. 190,88.

Price, K., Smith, S.E. (1971) Lancet i,130.

Pugh, C.E.M., Quastel, J. (1937) Biochem. J. 31,2306.

Ravaris, C.L., Nies, A., Robinson, D.S., Ives, J.O., Lamborn, K.R., Korson, L. (1976) Arch. Gen. Psychiat. 33,347.

Rebec, G.V., Groves, P.M. (1976) Pharmacol. Biochem. Behav. 5,349.

Reite, O.B. (1972) Physiol. Rev. 52,778.

Reynolds, G.P. (1979) Trends Neurosci. 2,265.

Reynolds, G.P., Ceasar, P.M., Ruthven, C.R.J., Sandler, M. (1978) Clin. Chim. Acta 84,225.

Reynolds, G.P., Gray, D.O. (1976) Clin. Chim. Acta 70,213.

Reynolds, G.P., Riederer, P., Sandler, M., Jellinger, K., Seemann, D. (1978) J. Neural. Transmiss. 43,271.

Riederer, P. Youdim, M.B.H., Rausch, W.D., Birkmayer, W., Jellinger, K., Seemann, D. (1978) J. Neural. Transmiss. 43,217.

Riederer, P., Reynolds, G.P. (1980) Brit. J. Clin. Pharmacol. 9,98.

Rinne, U.K., Siirtola, T., Sonninen, V. (1978) J. Neural Transmiss. 43,253.

Robinson, D.S., Lovenberg, W., Keiser, H., Sjoerdsma, A. (1968) Biochem. Pharmacol. 17,109.

Robinson, D.S., Nies, A., Ravaris, C.L., Lamborn, K.R. (1973) Arch. Gen. Psychiat. 29,407.

Rodriguez de Lores Arnaiz, G., De Robertis, E.D.P. (1962) J. Neurochem. 9,503.

Rosen, J.A. (1969) In: Progress in Neurogenetics (ed. Barbeau, A., Brunette, J.R.) Vol 1 p346 Int. Congr. Series No 175 Excerpta Medica Foundation, Amsterdam.

Roth, J.A. (1976) J. Neurochem. 27, 1107.

Roth, J.A., Feor, K. (1978) Biochem. Pharmacol. 27,1606.

Rothschild, Z., Schayer, R.W. (1958) Biochim. Biophys. Acta 30,23.

Saavedra, J.M., Axelrod, J. (1973) Proc. Nat. Acad. Sci. U.S.A. 70,769.

Sabelli, H.C., Borison, R.L., Diamond, B.I., Havdala, H.S., Narasimhachari, N. (1978a) Biochem. Pharmacol. 27,1707.

Sabelli, H.C., Borison, R.L., Diamond, B.I., May, J., Havdala, H.S. (1978b) In:Noncatecholic Phenylethylamines (ed. Mosnaim, A.D., Wolf, M.E.) part 1 p345 Dekker, New York.

Sabelli, H.C., Mosnaim, A.D. (1974) Amer. J. Psychiat. 131,695.

Sandler, M. (1977) In: Neurotransmission and Disturbed Behavior (ed. Van Praag, H.M., Bruinvels, J.) p150 Bonn, Scheltema, Holkema; Utrecht.

Sandler, M., Bonham Carter, S., Cuthbert, M.F., Pare, C.M.B. (1975) Lancet i,1045.

Sandler, M., Bonham Carter, S., Goodwin, B.L., Ruthven, C.R.J. (1976) In: Trace Amines and the Brain (ed. Usdin, E., Sandler, M.) p233 Dekker, New York.

Sandler, M., Glover, V., Ashford, A., Stern, G.M. (1978) J. Neural Transmiss. 43,209.

Satake, K. (1955) Sakinno Kosokazaku 4,39 cited by Okumoro, H. (1960) Bull. Osaka Med. Sch. 6,58.

Schayer, R.W., Cooper, J.A.D. (1956) J. App. Physiol. 9,481.

Schayer, R.W., Reilly, M.A. (1973) J. Pharmacol. Exp. Ther. 184,33.

Schildkraut, J.J. (1965) Amer. J. Psychiat. 122,509.

Schnaitman, C., Greenawalt, J.W. (1968) J. Cell Biol. 38, 158.

Schwab, R.S., England, A.C., Poskanzer, D.C., Young, R.R. (1969) J. Amer. Med. Ass. 208,1168.

Schwartz, M.A., Aikens, A.M., Wyatt, R.J. (1974a) Psychopharmacologia 38,319.

Schwartz, M.A., Wyatt, R.J., Yang, H.Y.T., Neff, N.H. (1974b) Arch. Gen. Psychiat. 31,557.

Schwartz, J.C. (1977) Ann. Rev. Pharmacol. Toxicol. 17,325.

Schwartz, J.C., Barbin, G., Baudry, M., Garbarg, M., Martres, M.P., Verdière, M. (1979) In: Current Developments in Psychopharmacology (ed. Essman, W.B., Valzelli, L.) Vol 5 p173 Spectrum, New York.

Schwartz, J.C., Lampart, C., Rose, C. (1970) J. Neurochem. 17,1527.

Schwartz, J.C., Pollard, H., Bischoff, S., Rehault, M.C., Verdiere-Sahuque, M. (1971) Eur. J. Pharmacol. 16,326.

Schwartz, J.C., Rose, C., Caillens, H. (1973) J. Pharmacol. Exp. Ther. 184,766.

Schweitzer, J.W., Friedhoff, A.J., Schwartz, R. (1975) Biol. Psychiat. 10,277.

Seakins, J.W.T. (1971) Clin. Chim. Acta 35,121.

Segal, D.S., Mandell, A.J. (1974) Pharmacol. Biochem. Behav. 2, 249

Senanayake, N., Vyravanathan, S., Kanagasuriyam, S. (1978) Brit. Med. J. 2,1127.

Severs, P.S., Caldwell, J., Williams, R.T. (1976) Psychol. Med. 6,35.

Shore, P.A., Cohn, V.H. (1960) Biochem. Pharmacol. 5,91.

Silberstein, S.D., Shein, H.M., Berv, K.R. (1972) Brain Res. 41,245.

Silkaitis, R.P., Mosnaim, A.D. (1976) Brain Res. 114,105.

Simpson, L.L. (1978) Biochem. Pharmacol. 27,1591.

Sjoerdsma, A., Lovenberg, W., Oates, J.A., Crout, J.R., Udenfriend, S., (1959a) Science 130,225.

Sjoerdsma, A., Oates, J.A., Zaltman, P., Udenfriend, S. (1959b) J. Pharmacol. Exp. Ther. 126,217.

Smith, C.K., Durack, D.T. (1978) Ann. Int. Med. 88,520.

Snyder, S.H. (1972) Arch. Gen. Psychiat. 27,169.

Snyder, S.H., Axelrod, J., Bauer, H. (1964) J. Pharmacol. Exp. Ther. 144,373.

Snyder, S.H., Brown, B., Kuhar, M.J. (1974) J. Neurochem. 23,37.

Snyder, S.H., Glowinski, J., Axelrod, J. (1966) J. Pharmacol. Exp. Ther. 153,8.

Solomon, P., Mitchell, R.S., Prinzmetal, M. (1937) J. Amer. Med. Ass. 108,1765.

Somerville, D. (1913) Proc. Roy. Soc. Med. 6,1.

Sourkes, T.L., Missala, K. (1976) In: Monoamine Oxidase and Its Inhibition (ed. Wolstenholme, G.E.W., Knight, J.) Ciba Foundation Symposium 39 p83 Elsevier, Amsterdam.

Squires, R.F. (1972) In: Monoamine Oxidase - New Vistas (ed. Costa, E., Sandler, M.) Adv. Biochem. Psychopharmacol. Vol. 5 p 355 Raven, New York.

Stern, F.H. (1963) J. Amer. Geriat. Soc. 11,670.

Stern, G.M. (1966) Brain 89,449.

Stern, G.M., Lees, A.J., Sandler, M. (1978) J. Neural Transmiss. 43,245.

Student, A.K., Edwards, D.J. (1977) Biochem. Pharmacol. 26,2337.

Suzuki, O., Katsumata, Y., Oya, M. (1979) Life Sci. 24,2227.

Suzuki, O., Yagi, K. (1976) Experientia 32, 13.

Sweet, R.D., McDowell, F.H. (1975) Ann. Int. Med. 83,456.

Taylor, K.M., Snyder, S.H. (1973) J. Neurochem. 21,1215

Tedeschi, D.H., Fellows, E.J. (1964) Science 144,1225.

Teychenne, P.F., Calne, D.B., Lewis, P.J., Findley, L.J. (1975) Clin. Pharmacol. Ther. 18,273.

Tipton, K.F. (1972) In: Monoamine Oxidase - New Vistas (ed. Costa, E., Sandler, M.) Adv. Biochem. Psychopharmacol. Vol. 5 p11 Raven, New York.

Tipton, K.F., Youdim, M.B.H. (1976) In: Monoamine Oxidase and Its Inhibition (ed. Wolstenholme, G.E.W., Knight, J.) Ciba Foundation Symposium 39 p393 Elsevier, Amsterdam.

Tozer, T.N., Neff, N.H., Brodie, B.B. (1966) J. Pharmacol. Exp. Ther. 153,177.

Tran., V.T., Chang, R.S.L., Snyder, S.H. (1978) Proc. Nat. Acad. Sci. U.S.A. 75,6290.

Trendelenburg, U., Draskóczy, P.R., Graefe, K.H. (1972) In: Monoamine Oxidase - New Vistas (ed. Costa, E., Sandler, M.) Adv. Biochem. Psychopharmacol. Vol 5 p371 Raven, New York.

Utena, H., Kanamura, H., Suda, S., Nakamura, R., Machiyama, R., Takahashi, R., (1968) Proc. Jap. Acad. 44,1078.

Van Slijke, L., Hart, B. (1903) Amer. Chem. J. 30,8.

Varga, E. (1965) Proc. Third Hungarian Conference for Therapy and Pharmacological Research, p197 Budapast.

Varga, E., Tringer, L. (1967) Acta Med. Acad. Sci. Hung. 23,289.

Verdière, M., Rose, C., Schwartz, J.C. (1974) Agents and Actions 4,184.

Vogel, W.H., Orfei, V., Century, B. (1969) J. Pharmacol. Exp. Ther. 165,195.

Waldmeier, P.C., Delini-Stula, A., Maître, L. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 292,9.

Waldmeier, P.C., Feldtrauer, J.J., Maître, L. (1977) J. Neurochem. 29,785.

Waldmeier, P.C., Felner, A.E. (1978) Biochem. Pharmacol. 27,801.

Wan, S.H., Matin, S.B., Azarnoff, D.L. (1978) Clin. Pharmacol. Ther. 23,585.

Weil-Malherbe, H. (1961) Methods Med. Res. 6,132.

Weil-Malherbe, H., Posner, H.S., Bowles, G.R. (1961) J. Pharmacol. Exp. Ther. 132,278.

White, H.L., Glassman, A.T. (1977) J. Neurochem. 29,987.

White, H.L., Wu, J.C. (1975) J. Neurochem. 25,21.

White, T. (1966) Brit. J. Pharmacol. 26,494.

Williams, D., Gascoigne, J.E., Williams, E.D. (1975) Histochem. J. 7,585.

Wong, K.P., Ruthven, C.R.J., Sandler, M. (1973) Clin. Chim. Acta 47,215.

Wu, P.H., Boulton, A.A. (1975) Canad. J. Biochem. 53,42.

Wyatt, R.J., Fram, D.H., Buchbinder, R., Snyder, F. (1971a) New Engl. J. Med. 285,987.

Wyatt, R.J., Fram, D.H., Kupfer, D.J., Snyder, F. (1971b) Arch. Gen. Psychiat. 24,145.

Yahr, M.D. (1978) J. Neural Transmiss. 43,227.

Yang, H.-Y.T., Neff, N.H. (1973) J. Pharmacol. Exp. Ther. 187,365.

Yang, H.-Y.T., Neff, N.H. (1974) J. Pharmacol. Exp. Ther. 189,733.

Youdim, M.B.H. (1972) In: Monoamine Oxidase - New Vistas (ed. Costa, E., Sandler, M.) Adv. Biochem. Psychopharmacol. Vol.5 p67 Raven, New York.

Youdim, M.B.H. (1976) In: Neuroregulators and Psychiatric Disorders (ed. Usdin, E., Hamburg, D.A., Barchas, S.D.) p57 Oxford University Press, New York.

Youdim, M.B.H., Aronson, J.K., Blau, K., Green, A.R., Grahame-Smith, D.G. (1979) Psychol Med. 9,377.

Youdim, M.B.H., Sandler, M. (1967) Biochem. J. 105, 43P.

Young, E., Scoville, W. (1938) Med. Clin. North Amer. 22,637.

Zappia, V., Zydek-Cwick, C., Schlenk, F. (1969) J. Biol. Chem. 244,4499.

Zeller, E.A. (1951) In: The Enzymes, Chemistry and Mechanism of Action (ed. Summer, J.B., Myrbäck, K.) Vol.2 p 536 Academic Press, New York.

Zeller, E.A. (1961) J. Neuropsychiat. 2,S1, 125.

Zeller, E.A. (1963) Ann. N.Y. Acad. Sci. 107,811.

Zeller, E.A., Barsky, J. (1952) Proc. Soc. Exp. Biol. Med. 81,459.

Psychopharmacology 57, 33–38 (1978)

Psychopharmacology
© by Springer-Verlag 1978

Deprenyl Administration in Man: A Selective Monoamine Oxidase B Inhibitor Without the 'Cheese Effect'

J. D. ELSWORTH[1], VIVETTE GLOVER[1], G. P. REYNOLDS[1], M. SANDLER[1,*], A. J. LEES[2], P. PHUAPRADIT[2], K. M. SHAW[2], G. M. STERN[2], and PARVEEN KUMAR[3]

[1] Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, London, W6 OXG, United Kingdom

[2] Department of Neurology, University College Hospital, London, WC1E 6AU, United Kingdom

[3] Department of Gastroenterology, St. Bartholomew's Hospital, London, EC1A 7BE, United Kingdom

Abstract. After pretreatment with the selective monoamine oxidase B inhibitor, (–)-deprenyl, in doses sufficient for complete inhibition of the platelet enzyme, 4 normal and 6 parkinsonian volunteers (2 receiving levodopa and 2 levodopa plus carbidopa) suffered no adverse pressor reaction ('cheese effect') after challenge with oral tyramine in amounts considerably greater than those likely to be encountered in a normal diet. Nor did the levodopa-deprenyl combination itself result in a pressor response. Normal human intestinal mucosa was shown predominantly to contain the deprenyl-insensitive A form of the enzyme, which presumably degraded administered tyramine in the deprenyl-treated volunteers; even those receiving the drug for prolonged periods manifested no 'cheese effect', suggesting that the A form remained uninhibited. Intestinal monoamine oxidase A was able to oxidise dopamine, whereas in human platelet or striatum the amine is a monoamine oxidase B substrate. Like tyramine, oral phenylethylamine challenge with amounts greater than those known to be present in a normal diet similarly gave rise to no adverse reaction in (–)-deprenyl-treated subjects; the reasons for this remain to be determined.

Key words: Monoamine oxidase inhibitor – Monoamine oxidase – 'Cheese effect' – Depression – Parkinson's disease – Levodopa – Phenylethylamine – Dopamine – Deprenyl

The ingestion of certain drugs and foodstuffs, particularly those such as cheese which contain tyramine, may provoke hypertensive crises and even death from cerebral haemorrhage in patients undergoing treatment with monoamine oxidase (MAO) inhibitors (Marley and Blackwell, 1970). Because of this adverse reaction, which has been termed the 'cheese effect', the use of this group of drugs in psychiatric practice has been restricted. When multiple forms of MAO, with different substrate specificities, were described (see Sandler and Youdim, 1972), the possibility of tailoring specific inhibitors, selectively to prevent the oxidation of some monoamines while allowing the degradation of others (particularly tyramine) to proceed, became feasible (Youdim et al., 1971).

In recent years, a classification of the different types of MAO into A and B forms (Johnston, 1968) has gained widespread acceptance. This classification was, in fact, made possible by the synthesis of, and is based on the response of the enzyme to the selective inhibitor, clorgyline. Thus, MAOA is defined as that form of the enzyme sensitive to clorgyline, which preferentially oxidises the 'neurotransmitter monoamines', 5-hydroxytryptamine and noradrenaline (Johnston, 1968); MAOB is resistant to clorgyline and, in general, prefers phenylethylamine as substrate (Yang and Neff, 1973). In human platelets and brain, dopamine is oxidised by MAOB (Glover et al., 1977a). The extent to which it is oxidised by MAOA in various human tissues is still unclear (Glover et al., 1977a). Tyramine appears to be a substrate for both MAOA and B; even so, the expectation that its in vivo oxidation in man would proceed after clorgyline administration was not borne out during experiments in a group of volunteers (Lader et al., 1970).

The selective MAOB inhibitor (–)-deprenyl has been studied in Hungary for more than ten years (Knoll, 1976), and preliminary clinical reports (Varga and Tringer, 1967; Tringer et al., 1968) indicate that it may have a beneficial effect in depressive illness. The active (–)-isomer has only very recently become avail-

* *Address for offprint requests:* Professor Merton Sandler, Queen Charlotte's Maternity Hospital, Goldhawk Road, London, W6 OXG, United Kingdom

0033-3158/78/0057/0033/$ 1.20

able for clinical trial in the West, but early indications (Birkmayer et al., 1977; Lees et al., 1977) are that it has an adjuvant role in the treatment of parkinsonism when administered with levodopa and a peripheral decarboxylase inhibitor. A most important potential property attributed to (–)-deprenyl is that its administration is not associated with the 'cheese effect' (Knoll, 1976). This claim was based largely on extrapolation from preliminary data (Squires, 1972) indicating that human intestinal mucosal MAO is of the A variety and would therefore continue to metabolise ingested tyramine. It therefore seemed necessary to investigate this problem systematically in both normal and parkinsonian human volunteers by challenging them with increasing oral doses of tyramine and phenylethylamine before and during treatment with (–)-deprenyl. Parallel observations were also made on normal human intestinal mucosal MAO, obtained during the course of diagnostic biopsy, in an attempt to confirm and extend the earlier findings (Squires, 1972).

MATERIALS AND METHODS

Tests involving the oral administration of tyramine (Lader et al., 1970) and phenylethylamine were carried out on four normal male volunteers and six patients (four males aged 38–62 years and two females aged 71 and 72 years) with Parkinson's disease, following their informed consent. The study was accepted by the University College Hospital and Medical School Committee on the Ethics of Clinical Investigations and was performed in hospital by an experienced physician with full resuscitative measures available. Tyramine and phenylethylamine-containing foods were forbidden for one week prior to (–)-deprenyl administration, at the end of which time an oral amine test was carried out. Solutions of the amine hydrochloride in water were used for the tests. (–)-Deprenyl was started the following day, its dosage gradually increasing over one week (Fig. 1) to 10 mg daily. The oral amine test was then repeated in all volunteers and again in four of the parkinsonians after two months' continuous (–)-deprenyl treatment. The interval between tyramine and phenylethylamine tests was between 2 and 7 days for the patients. As recovery of the normal volunteer's platelet MAO activity from deprenyl inhibition was being monitored, however, drug administration was stopped after the first amine test and then restarted for the second test after a period of at least 3 weeks.

The test was carried out in an unblind fashion as follows: with the subject resting in bed and fasting, progressively doubled doses of tyramine and phenylethylamine were given at 30–45-min intervals, commencing with 25 mg tyramine hydrochloride or 6.5 mg phenylethylamine hydrochloride for the control test, and 6.25 mg tyramine hydrochloride or 0.65 mg phenylethylamine hydrochloride when the subjects were taking (–)-deprenyl. Pulse and blood pressure were measured every 5 min. Tests were terminated when heart rate slowed by 10 beats/min, or when a systolic blood pressure rise of 20 mg Hg occurred, or if previously agreed maximum doses of tyramine hydrochloride (400 mg) or phenylethylamine hydrochloride (26 mg) were reached while the subject was receiving (–)-deprenyl.

Blood and urine samples were obtained before, during, and after (–)-deprenyl treatment. The harvesting of platelets was performed as previously described (Glover et al., 1977b). Three samples of blood were obtained from the volunteers while they were taking (–)-deprenyl, being drawn 2, 5, and 10 h after drug ingestion. Recovery of activity was monitored from single samples taken at the same time of day as the 5-h post-drug sample. Urine was collected every 3 h during chosen days, preserved by 3 ml 6 N HCl and stored at –20° C until assay. Free phenylethylamine content of urine samples was measured gas chromatographically (Reynolds et al., unpublished), a process involving the extraction of phenylethylamine from urine at pH 12 into hexane and subsequent back-extraction into 1 M HCl. The dried amine fraction was then derivatised with pentafluorobenzoyl chloride and, after evaporation of excess reagent, injected in ethyl acetate into a gas chromatograph equipped with an electron capture detector. Amine separation was achieved on a 6-ft column of 2.5% OV 225 at 210° C. *p*-Methylphenylethylamine added to the urine before extraction served as an internal standard for quantification.

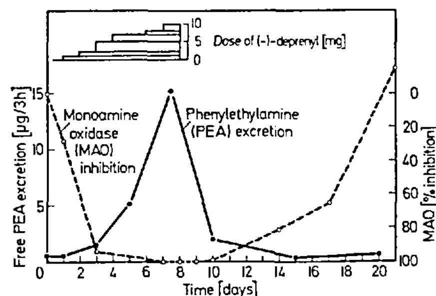


Fig. 1. Typical changes in platelet monoamine oxidase activity and free urinary phenylethylamine output in normal volunteer receiving (–)-deprenyl

Biopsy samples (1–2 mg) of human jejunal tissue obtained for diagnostic purposes and found to be normal were placed immediately on dry ice and stored at –20° C. Samples were mechanically homogenised just before assay to give a 10% (w/v) homogenate in 0.1 M phosphate buffer, pH 7.4, and in some experiments were preincubated with (–)-deprenyl as before (Glover et al., 1977a). MAO was assayed as described previously (Glover et al., 1977a, b), with the following modifications: final substrate concentrations in incubation mixtures were tyramine 150 µM, dopamine 300 µM, 5-hydroxytryptamine 300 µM, and phenylethylamine 30 µM, and the specific activity of phenylethylamine was increased 5-fold to give higher sample-blank ratios. Higher concentrations of dopamine and 5-hydroxytryptamine were employed to approach saturating concentrations and to give higher activities. Protein was assayed by Lowry's method (Lowry et al., 1951).

RESULTS

A typical MAO inhibitory response to (–)-deprenyl in one of the normal volunteers is shown in Figure 1. In all four, and also in the patients, platelet activity was completely absent by the time the dose had reached 10 mg daily. In 3 out of the 4 volunteers, a decline in platelet MAO activity was observed 2 h after taking 1 mg (–)-deprenyl. The recovery rate varied from subject to subject, although in all, activity was still absent on the day after (–)-deprenyl administration ceased.

Urinary phenylethylamine output was monitored in the volunteers and a typical response is shown in

Figure 1. Concentration was not altered by 1 mg (–)-deprenyl, but rose strikingly as the dose was increased, falling sharply after the drug was withdrawn. The 10-mg dose caused a 20 to 90-fold rise in phenylethylamine excretion in the volunteers compared with their control values. The return to normal output was more rapid than the reestablishment of platelet MAO activity.

Before (–)-deprenyl, the four normal volunteers all reached the 400-mg dose of tyramine hydrochloride (cumulatively 775 mg) before any rise in blood pressure occurred. Certain clinical counterparts of the pressor response were observed, including forceful heartbeat, sweating, mild apprehension, and pallor. After 1 week of (–)-deprenyl, three of the volunteers reached a dose of 200 mg (cumulatively 375 mg) before their blood pressure rose, while the fourth continued to tolerate a dose of 400 mg (Table 1). Feelings of well-being, increased energy, and 'light-headedness' were reported by the volunteers during (–)-deprenyl treatment. Phenylethylamine challenge before (–)-deprenyl with doses up to 130 mg of hydrochloride (245 mg cumulatively) did not provoke a pressor response. At this stage, however, one volunteer experienced a mild, delayed amphetamine-like reaction. The response involved tachycardia (120 beats/min), ectopic pulse beats, dilated pupils, and feelings of mild apprehension and increased energy. When taking (–)-deprenyl with phenylethylamine, however, this subject had no such response.

Table 1. Responses of healthy normal volunteers to oral tyramine

Volunteer	Dosage of tyramine hydrochloride at which response occurred (mg)		Pulse fall (beats/min)	Blood pressure rise (systolic/diastolic) (mm Hg)
1 M	Before deprenyl	400	10	28/0
	On 10 mg deprenyl	200	14	31/0
2 M	Before deprenyl	400	0	30/8
	On 10 mg deprenyl	400	0	22/6
3 M	Before deprenyl	400	20	40/3
	On 10 mg deprenyl	200	11	22/0
4 M	Before deprenyl	400	12	25/5
	On 10 mg deprenyl	200	16	20/10

The six patients with idiopathic Parkinson's disease included two previously untreated cases, two receiving levodopa and two levodopa and carbidopa. Tyramine tolerance before deprenyl was sometimes smaller in this group than in the normal volunteers, but the response to deprenyl was similar (Table 2). Lability of blood pressure in levodopa-treated patients made assessment difficult, as did the absence of bradycardia in some cases (Table 2). No decrease in tyramine tolerance occurred in four of the patients after two month's (–)-deprenyl therapy at a dose of 10 mg daily. One untreated case developed a pressor response to 65 mg phenylethylamine hydrochloride, which was rapidly reversed by 5 mg phentolamine i.v. No other effects were seen after phenylethylamine challenge and doses of 26 mg of the amine hydrochloride were tolerated during deprenyl treatment without ill-effect.

Table 2. Responses of patients with Parkinson's disease to oral tyramine

Patient (age)	Antiparkinsonian treatment (daily dose)	Dosage of tyramine hydrochloride at which response occurred (mg)		Pulse fall (beats/min)	Blood pressure rise (systolic diastolic) (mm Hg)
1 M (52)	None	before deprenyl	400	0	30/5
		1 week 10 mg deprenyl	200	0	25/0
		8 weeks 10 mg deprenyl	200	0	30/5
2 F (72)	None	before deprenyl	200	10	15/5
		1 week 10 mg deprenyl	200	12	10/0
3 F (71)	Levodopa (3.5 g)	before deprenyl	200	0	20/8
		1 week 10 mg deprenyl	400	0	5/0
		8 weeks 10 mg deprenyl	200	0	10/5
4 M (38)	Levodopa (3.0 g)	before deprenyl	400	6	30/10
		1 week 10 mg deprenyl	200	0	40/5
5 M (54)	Levodopa plus carbidopa (500 mg)	before deprenyl	400	12	40/5
		1 week 10 mg deprenyl	400	15	35/5
		8 weeks 10 mg deprenyl	400	21	50/5
6 M (62)	Levodopa plus carbidopa (900 mg)	before deprenyl	400	12	45/15
		1 week 10 mg deprenyl	150	20	50/20
		8 weeks 10 mg deprenyl	150	24	45/15

Table 3. Comparison of human intestinal and platelet MAO activities with different substrates

	Intestine ($N = 13$)	Platelet ($N = 5$)
Tyramine	60.5 ± 6.6 (100)	15.0 ± 0.9 (100)
Dopamine	62.4 ± 1.3 (100.1)	14.9 ± 2.4 (97.2)
5-Hydroxytryptamine	55.9 ± 11.5 (89.2)	< 2
Phenylethylamine	7.8 ± 10.2 (12.6)	6.3 ± 0.23 (42.1)

Activities are expressed as nmoles of substrate oxidised/mg protein/30 min ± SEM. Figures in parentheses represent percentages of different activities with respect to tyramine values

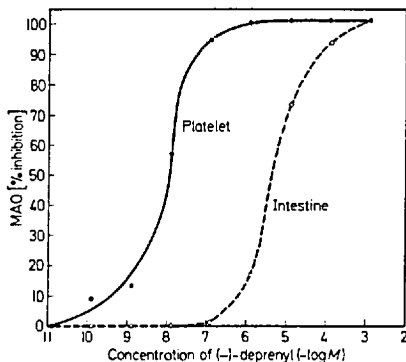


Fig. 2. Comparison of (-)-deprenyl inhibition of tyramine oxidase by human intestinal mucosa (*each point*: mean of 3 samples) and phenylethylamine oxidase by human platelet (*each point*: mean of 2 samples). Concentration of (-)-deprenyl is that in final reaction mixture. Samples were preincubated with (-)-deprenyl for 30 min at room temperature before assay

Table 3 shows mean MAO activity of human intestinal mucosal biopsy samples with several substrates. There is considerably greater activity with 5-hydroxytryptamine, dopamine, and tyramine than with phenylethylamine. Figure 2 shows the inhibitory action of (-)-deprenyl on tyramine oxidation by gut mucosa and phenylethylamine degradation by platelet MAO. Intestinal activity is about one thousand times less sensitive to (-)-deprenyl inhibition than that of the platelet enzyme. Single experiments on the inhibition of intestinal dopamine- and 5-hydroxytryptamine-oxidising activity by (-)-deprenyl showed a similar pattern to that with tyramine.

DISCUSSION

Evidence is beginning to emerge of a particular role for (-)-deprenyl in the treatment of Parkinson's disease (Birkmayer et al., 1977; Lees et al., 1977). Early clinical impressions of a therapeutic response to the drug in depressive illness (Varga and Tringer, 1967; Tringer et al., 1968) await confirmation, but if substantiated they will shed light on the nature of the putative disturbance of monoamine systems in this disease; whereas earlier theories centered on a possible deficit of noradrenaline (Schildkraut, 1965) or 5-hydroxytryptamine (Lapin and Oxenkrug, 1969), neither amine is a substrate for MAOB, which appears to be selectively inhibited by (-)-deprenyl (Knoll, 1976). The present series of experiments show that inhibition of platelet MAO can be achieved extremely rapidly, and the beneficial effect in depression, it was claimed, appeared equally rapidly. This drug response contrasts with that observed with a conventional MAO inhibitor such as isocarboxazid (Robinson et al., 1968), where the delay before clinical benefit supervened was matched by a correspondingly slow decrease in platelet activity. Unlike (-)-deprenyl, these drugs seem unable to abolish activity completely.

The lightening of affect produced by (-)-deprenyl, if confirmed, may well be associated with a build-up of some other monoamine substrates of MAOB, either dopamine, or perhaps phenylethylamine, both of which have been invoked as the bases of alternative hypotheses for the pathogenesis of depressive illness (Randrup et al., 1975; Sabelli and Mosnaim, 1974). It may not be without significance, therefore, that the urinary output of phenylethylamine rose steeply in the normal volunteers at a time when complete MAOB inhibition may be presumed to have taken place (Fig. 1). Indeed, we believe that we are entitled to employ this test as a clinical index of selective MAOB inhibition, just as urinary tryptamine output (Sjoerdsma et al., 1959) was formerly used as a guide to overall inhibition of the enzyme.

Our in vivo data correspond well with the in vitro results: if we calculate that 10 mg of (-)-deprenyl might be evenly distributed in an average human water space of 40 kg, it would correspond approximately to about a 10^{-6} M concentration, which would probably be sufficient largely to inhibit brain MAOB (Glover et al., 1977a). Figure 2 indicates that platelet MAO should be completely inhibited at this concentration, whereas at a dose of 1 mg inhibition would be less complete. The 1–10-mg dosage range corresponds with the observed partial to total platelet MAO inhibition (Fig. 1). At these same concentrations, the tyramine-oxidising ability of the intestinal enzyme is comparatively unaffected (Fig. 2). There is little activity towards phenylethylamine in this tissue, reflecting a

relative absence of MAOB. Therefore, tyramine degradation carried out by MAOA may proceed vigorously even after MAOB activity has been completely inhibited by (–)-deprenyl. It should be noted, though, that other tissues as well as the intestine may play a role in the degradation of oral tyramine.

These in vitro findings may explain the observation that substantial amounts of tyramine, considerably larger than those likely to be encountered in a diet containing even the most generous quantities of cheese, could be eaten with impunity with the doses of (–)-deprenyl employed (Tables 1 and 2). The results contrast sharply with those of others following administration of the selective MAOA inhibitor clorgyline, when small doses of tyramine only were sufficient to produce a profound pressor response (Lader et al., 1970). In view of the fact that levodopa (Sandler, 1972), or foods that contain it, also provoke a severe hypertensive reaction when administered with conventional MAO inhibitors, it is interesting that the two parkinsonian patients treated with a (–)-deprenyl-levodopa combination experienced no untoward pressor effects (Table 2). Dopamine is predominantly degraded by MAOB in platelet and striatum (Glover et al., 1977a), but is vigorously metabolised by the MAOA of the intestinal mucosa (Table 3). Therefore, perhaps dopamine generated in the gut plays an important role in the hypertensive adverse reaction if it is not inactivated locally; alternatively, substantial peripheral sources of MAOA with dopamine-oxidising ability may exist, other than the gut.

Phenylethylamine is also present in the diet (Sandler et al., 1974; Schweitzer et al., 1975), even though concentrations are not as great as those in tyramine-containing foods. Experimental animals given a combination of MAO inhibitor and phenylethylamine manifest an amphetamine-like response (Mantegazza and Riva, 1963), as might be predicted from the similarity of phenylethylamine to amphetamine (Sandler and Reynolds, 1976). But these studies employed much higher doses than those in the tests reported here. Nevertheless, the drug combination obviously forms a potential hazard, so that it seemed important to assess the response to this amine after (–)-deprenyl pretreatment. Despite the adverse reaction to 130 mg phenylethylamine hydrochloride in one subject prior to (–)-deprenyl, which was probably idiosyncratic, oral phenylethylamine alone seems to be safe in most individuals; very large amounts have been ingested (Chen, 1927; Seakins, 1971) without deleterious effect. There were no untoward reactions after graded phenylethylamine dosage up to amounts well in excess of likely dietary intake in (–)-deprenyl-pretreated subjects. Recent evidence points to the existence of substantial phenylethylamine-oxidising ability distributed in a widespread amine oxidase system distinct from classical MAO (Lewinsohn et al., unpublished). (–)-Deprenyl is an irreversible MAOB inhibitor (Knoll, 1976), but it exerts its selective action only within a certain concentration range (Fig. 2). Higher doses also inhibit MAOA so that it is possible that the selective effect is observed only during acute dosage; prolonged administration might conceivably exert a cumulative inhibitory effect on MAOA. However, continued treatment of four parkinsonian patients with (–)-deprenyl for a period of two months did not abolish their freedom from the 'cheese effect' after challenge with high oral doses of tyramine. The explanation of this may lie in the rate of turnover of MAO, the rate of breakdown of (–)-deprenyl, or as has been shown in rat liver at least, in the fact that inhibition of MAOA by high concentrations of (–)-deprenyl is reversible while that of MAOB is irreversible (Egashira et al., 1976).

The claim of Knoll (Knoll, 1976) that deprenyl administration is free from the 'cheese effect' has now received substantial experimental backing and the drug may thus find important applications in clinical practice.

Acknowledgements. We are grateful to Professor J. Knoll and to the Chinoin Chemical Co., Budapest, for generous supplies of (–)-deprenyl. J.D.E. is supported by a grant from the Parkinson's Disease Society, V.G. by a grant from the Migraine Trust, and G.P.R. by the Schizophrenia Research Fund.

REFERENCES

Birkmayer, W., Riederer, P., Ambrozi, L., Youdim, M. B. H.: Implications of combined treatment with 'madopar' and L-deprenil in Parkinson's disease. Lancet 1977 I, 439–443.

Chen, K. K.: A comparative study of ephedrine, pseudoephedrine and β-phenylethylamine with reference to their effects on the pupil and on the blood pressure. Arch. Int. Med. 39, 404–411 (1927)

Egashira, T., Ekstedt, B., Oreland, L.: Inhibition by clorgyline and deprenyl of the different forms of monoamine oxidase in rat liver mitochondria. Biochem. Pharmacol. 25, 2583–2586 (1976)

Glover, V., Sandler, M., Owen, F., Riley, G. J.: Dopamine is a monoamine oxidase B substrate in man. Nature 265, 80–81 (1977a)

Glover, V., Sandler, M., Grant, E., Rose, F. C., Orton, D., Wilkinson, M., Stevens, D.: Transitory decrease in platelet monoamine oxidase activity during migraine attacks. Lancet 1977b I, 391–393

Johnston, J. P.: Some observations upon a new inhibitor of monoamine oxidase in brain tissue. Biochem. Pharmacol. 17, 1285–1297 (1968)

Knoll, J.: Analysis of the pharmacological effects of selective monoamine oxidase inhibitors. In: Monoamine oxidase and its inhibition, G. E. W. Wolstenholme and J. Knight, eds., pp. 135–161. Amsterdam: Elsevier 1976

Lader, M. H., Sakalis, G., Tansella, M.: Interactions between sympathomimetic amines and a new monoamine oxidase inhibitor. Psychopharmacologia (Berl.) 18, 118–123 (1970)

Lapin, I. P., Oxenkrug, G. K.: Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. Lancet 1969 I, 132–136

Lees, A. J., Shaw, K. M., Kohout, L. J., Stern, G. M., Elsworth, J. D., Sandler, M., Youdim, M. B. H.: Deprenyl in Parkinson's disease. Lancet 1977 II, 791–796.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265–275 (1951)

Mantegazza, P., Riva, M. J.: Amphetamine-like activity of β-phenylethylamine after a monoamine oxidase inhibitor *in vitro*. J. Pharm. Pharmacol. **15**, 472–478 (1963)

Marley, E., Blackwell, B.: Interactions of monoamine oxidase inhibitors, amines and foodstuffs. Adv. Pharmacol. Chemother. **8**, 186–239 (1970)

Randrup, A., Munkvad, I., Fog, R., Gerlach, J., Molander, L., Kjellberg, B., Scheel-Kruger, J.: Mania, depression and brain dopamine. In: Current developments in psychopharmacology, vol. 2, W. B. Essman and L. Valzelli, eds., pp. 205–248. New York: Spectrum 1975

Robinson, D. S., Lovenberg, W., Keiser, H., Sjoerdsma, A.: Effects of drugs on human blood platelets and plasma amine oxidase activity *in vitro* and *in vivo*. Biochem. Pharmacol. **17**, 109–119 (1968)

Sabelli, H. C., Mosnaim, A. D.: The phenylethylamine hypothesis of affective behaviour. Am. J. Psychiatry **131**, 695–699 (1974)

Sandler, M.: Catecholamine synthesis and metabolism in man (with special reference to parkinsonism). In: Handbook of experimental pharmacology, vol. 33, Catecholamines, H. Blaschko, and E. Muscholl, eds., pp. 845–899. Berlin: Springer 1972

Sandler, M., Youdim, M. B. H.: Multiple forms of monoamine oxidase: functional significance. Pharmacol. Rev. **24**, 331–348 (1972)

Sandler, M., Youdim, M. B. H., Hanington, E.: A phenylethylamine oxidising defect in migraine. Nature **250**, 335–337 (1974)

Sandler, M., Reynolds, G. P.: Does phenylethylamine cause schizophrenia? Lancet 1976 I, 70–71

Schildkraut, J. J.: The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry **122**, 509–522 (1965)

Schweitzer, J. W., Friedhoff, A. J., Schwartz, R.: Chocolate, β-phenylethylamine and migraine re-examined. Nature **257**, 256 (1975)

Seakins, J. W. T.: The determination of urinary phenylacetylglutamine as phenylacetic acid: studies on its origin in normal subjects and children with cystic fibrosis. Clin. Chim. Acta **35**, 121–131 (1971)

Sjoerdsma, A., Oates, J. A., Zaltzman, P., Udenfriend, S.: Identification and assay of urinary tryptamine: application as an index of monoamine oxidase inhibition in man. J. Pharmacol. Exp. Ther. **126**, 217–222 (1959)

Squires, R. F.: Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: a comparison of eight mammalian species. In: Monoamine oxidases—new vistas, Adv. Biochem. Psychopharmacol., vol. 5, E. Costa and M. Sandler, eds., pp. 355–370. New York: Raven 1972

Tringer, L., Haits, G., Varga, E.: The effect of L-E-250 (L-phenylisopropylmethyl-propinylamine-HCl) in depressions. Proc. V Conf. Hung. Ther. Invest. Pharmacol. (Soc. Pharmacol. Hung.), 111–114 (1968)

Varga, E., Tringer, L.: Clinical trial of a new type of promptly acting psychoenergetic agent (phenyl-isopropylmethyl-propinylamine-HCl, E-250). Acta Med. Acad. Sci. Hung. **23**, 289–295 (1967)

Yang, H.-Y. T., Neff, N. H.: β-Phenylethylamine: a specific substrate for type B monoamine oxidase of brain. J. Pharmacol. Exp. Ther. **187**, 365–371 (1973)

Youdim, M. B. H., Collins, G. G. S., Sandler, M.: Monoamine oxidase: multiple forms and selective inhibitors. Biochem. J. **121**, 34P–36P (1971)

Received August 26, 1977

DEPRENYL IN PARKINSON'S DISEASE

A. J. LEES K. M. SHAW
L. J. KOHOUT G. M. STERN

Department of Neurology, University College Hospital, London WC1E 6AU

J. D. ELSWORTH M. SANDLER

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynæcology, Queen Charlotte's Maternity Hospital, London W6 0XG

M. B. H. YOUDIM

Department of Pharmacology, School of Medicine, Israel Institute of Technology, Haifa, Israel

Summary In a double-blind crossover trial, (−)-deprenyl, a fast-acting selective mono-amine-oxidase-B inhibitor without a "cheese effect", was given to 41 patients with idiopathic Parkinson's disease who were receiving maximum tolerated doses of levodopa either alone or combined with carbidopa ('Sinemet'). In a dose of 10 mg, daily or on alternate days, (−)-deprenyl prolonged the therapeutic effect of levodopa and was effective in mild "on-off" disabilities with end-of-dose akinesia; the majority of patients with nocturnal and early-morning akinesia also improved. No

statistically significant improvement occurred in diurnal akinesia, and there was no improvement in patients with severe on-off disabilities with freezing and rapid oscillations ("yo-yo" effect).

Levodopa-induced dyskinesias were aggravated in 14 patients. In 5 previously untreated patients, (—)-deprenyl alone gave no benefit, but when it was used with levodopa and carbidopa a mean dosage reduction of 200 mg levodopa daily was possible. Depression, present in 15 patients, was unchanged. (—)-Deprenyl in combination with smaller total daily doses of levodopa and a peripheral decarboxylase inhibitor may prove useful in reducing the frequency and severity of some types of on-off effect with overall benefit comparable to that obtained with larger doses of levodopa.

Introduction

MONOAMINE oxidase (M.A.O.) is found in high concentration in the brain and plays an important part in the intraneuronal breakdown of dopamine. The discovery of nigrostriatal dopamine deficiency in Parkinson's disease led to the use of M.A.O. inhibitors in an attempt to increase central dopamine stores.[1] Clinical trials showed, however, that these agents had only weak antiparkinsonian effects when used alone[1,2] but that they potentiated the effects of levodopa.[1-3] Unfortunately, because of hypertensive crises this potentially valuable combination could not be used.[4]

M.A.O. is now known to exist in several forms which have different substrate specificities. They have been classified into two types, A and B. Type A oxidatively deaminates 5-hydroxytryptamine and noradrenaline and is selectively inhibited by clorgyline.[5] Type B deaminates phenylethylamine and benzylamine and is selectively inhibited by (—)-deprenyl,[6] the (—)-form of the compound being the active species. Tyramine and, to a variable extent, dopamine are known to be substrates for both forms of M.A.O. Recent work, however, has shown dopamine to be a B substrate in human brain,[7] whereas in rat brain it is oxidised by M.A.O.A.[8]

(—)-Deprenyl (phenylisopropylmethylpropinylamine hydrochloride) is a potent irreversible inhibitor of human platelet M.A.O.[9] Dietary restrictions are unnecessary, and it can be used safely with levodopa.[9] Birkmayer et al.,[10] using (—)-deprenyl (10 mg) daily, reported a 56% improvement in akinesia and relief of "on-off" effects in 223 patients receiving submaximum doses of levodopa plus benserazide ('Madopar'). In the present study, certain therapeutic effects of (—)-deprenyl have been observed in patients with Parkinson's disease already receiving maximum tolerated doses of levodopa alone or with carbidopa. Its effects on on-off effects and waning levodopa responses have been studied, and its antiparkinsonian properties have been examined in previously untreated patients.

Patients and Methods

41 patients with idiopathic Parkinson's disease agreed to participate in a double-blind crossover trial. They were a selected group only in that they were known to be observant individuals with attentive families. All were receiving maximum doses of levodopa, alone or with a peripheral dopa-decarboxylase inhibitor ('Sinemet'). All had benefited from levodopa initially but were now losing ground. 5 previously untreated patients were also studied.

Patients were classified into those with conspicuous and disabling diurnal fluctuations (on-off effects) (group 1) and those whose disabilities were fairly constant throughout the day (group 2). Group-1 patients were then observed in hospital for a day to confirm the extent of their disability. They were dressed, ambulant, and taking their meals and treatment at customary times. Akinesia and dyskinesia scores were made hourly on a 7-point scale, and patients were instructed in the use of a self-scoring diary. Attempts were made to clarify differences between tremor, dyskinesia, cramps, and dystonia, and the patients were advised to contact the assessor should scoring problems occur. At the end of the hospital day, patients were asked whether the hospital assessment gave an accurate presentation of their usual performance. The severity of on-off disabilities was graded according to a classification proposed by Barbeau:[11]

Grade of severity of on-off effects	*No. of cases*
I	1
2	4
3	11
4	6

Patients were given (—)-deprenyl 5 mg b.d. (run-in period) and were then seen every 2 weeks as outpatients. The self-scoring diaries were collected at each attendance and discussed with the patient and his family. After a month's deprenyl treatment, patients were readmitted to hospital for a further ward assessment, and the run-in period then ceased. At varying intervals after a month's continuous therapy, placebo was substituted at a time unknown to patient or assessor and continued for a month before active treatment was reinstated. At the end of the trial, those patients who had benefited from (—)-deprenyl were further assessed on the effect of adjusting the dose and the consequence of alternate-day administration.

Group-2 patients were then evaluated, at each visit, on the Columbia and North Western University disability scales, which were supplemented by computerised tracking and writing techniques. The 5 previously untreated patients were initially given (—)-deprenyl. Placebo was introduced in a double-blind manner, and the antiparkinsonian effect of (—)-deprenyl was measured. Levodopa-plus-carbidopa was then added to maximum tolerated doses or until no disability remained. Placebo for (—)-deprenyl was then given in a single-blind manner, and levodopa requirements were reassessed. In view of the possible antidepressant properties of (—)-deprenyl, the Zung self-rating depression scale was used to assess mood changes and emotional state. Serial platelet-M.A.O. activity was measured, with labelled phenylethylamine as substrate.[12] Other investigations included full blood-count, blood urea and electrolytes, liver-function tests, and electrocardiogram. These tests were repeated after 3 and 6 months of (—)-deprenyl treatment.

TABLE I—CLINICAL FEATURES OF THE PATIENTS

Group	No. of patients	Sex	Age (yr) (mean and range)	Age (yr) at time of diagnosis (mean and range)	Mean duration of levodopa treatment (yr)	Levodopa alone: No. of patients	Levodopa alone: Mean dosage (g)	Levodopa plus carbidopa: No. of patients	Levodopa plus carbidopa: Mean dosage (mg)
1	13	M	58	47	5·0	7	3·90	16	830
	10	F	(38–74)	(31–64)					
2	10	M	65	56	5·4	3	3·25	16	570
	9	F	(56–78)	(44–66)					

Results

The clinical features characterising patients in groups 1 and 2 are shown in table I. Both had received levodopa for a mean period of 5 years. Those with on-off disability, however, were on average 7 years younger and had an earlier age of onset of the disease than the group-2 patients.

Table II shows the frequency of the different types of on-off disturbance and the effects of (—)-deprenyl rated by means of physician's assessment during the day of admission and self-scoring diaries. 12 patients considered their ward assessment gave an optimistic assessment of their usual abilities, and diaries only were used in this group. End-of-dose akinesia, the most common pattern of fluctuation, improved in 12 out of 19 patients (65%), whereas freezing episodes, which in our patients were the most incapacitating disability, were not helped at all. Nocturnal and early-morning akinesia occurred with equal frequency in groups 1 and 2, and approximately two-thirds of the patients benefited.

TABLE II—PREVALENCE OF PATTERNS CONTRIBUTING TO ON-OFF DISTURBANCES AND RESPONSES TO (—)-DEPRENYL

Akinetic disabilities			Dyskinetic disabilities		
Disability	No. affected	No. improved	Disability	No. affected	No. improved
Rapid oscillations ("yo-yo" effects)	8	2	Peak dose	22	0
End-of-dose akinesia	19	12	End-of dose dyskinesia	1	0
Freezing episodes (rigid or hypotonic)	14	0	Early-morning dystonia	2	0
Early-morning akinesia	16	9			
Nocturnal akinesia	13	9			

Some of the patients with mild on-off disabilities were greatly improved, as illustrated by the following case-report:

In 1973 a 64-year-old woman with a 2-year history of right hemiparkinsonism started levodopa plus carbidopa, 250 mg t.d.s., with striking benefit. Within 9 months she began to experience peaks and troughs of performance and mild limb dyskinesias. Occasionally, levodopa brought no benefit but left her stiff and immobile, and at other times fatigue or emotional stress greatly reduced the duration of the levodopa effect. Because delays led to prolonged akinesia, precise timing of individual doses became crucial, and in 1976 she began taking 100 mg levodopa every 3 hours. By 1977, despite this closer spacing of her treatment, she was forced to compress her housework and meals into the first 2 hours after each dose of levodopa. The addition of (—)-deprenyl abolished this end-of-dose akinesia within 2 days of treatment, prolonged the effect of each levodopa dose to 4 hours, and allowed a daily dose reduction of 150 mg levodopa in the run-in period. Difficulty in turning in bed at night and morning stiffness were also improved. In the accompanying figure this patient's favourable response is compared with the failure of (—)-deprenyl to help another patient with "yo-yo" oscillations.

Comparison of (A) abolition by (—)-deprenyl of end-of-dose deterioration in one patient and (B) failure of (—)-deprenyl to modify the more severe yo-yo oscillations in another patient.

Table III shows the mean daily akinesia and dyskinesia scores in group-1 patients. The daily scores abstracted from the patients' diaries were summated and divided by the total number of days of treatment. There was no statistically significant improvement, a finding which may be explained by the frequent freezing attacks experienced by many of the patients. Similarly, in group 2 no significant improvement in disability scores occurred with (—)-deprenyl (table IV). In group-1 patients, "off" periods were distributed equally through the mornings, afternoons, and evenings.

At the onset of the trial, 15 of the 46 patients were rated as moderately or severely depressed on the Zung

TABLE III—AKINESIA AND DYSKINESIA SCORES IN GROUP-1 PATIENTS (ON-OFF EFFECTS)

Group 1 (22 patients)	Drug regimen	No. of patients	Mean disability score* (±S.E.)		Significance P
			(—)-Deprenyl	Placebo	
Akinesia	Levodopa plus carbidopa	16	6·58 (±1·07)	8·05 (±1·06)	<0·1
	Levodopa alone	6	10·81 (±2·09)	10·57 (±2·03)	N.S.
Dyskinesia	Levodopa plus carbidopa	16	11·30 (±1·46)	9·37 (±1·42)	<0·1
	Levodopa alone	6	9·43 (±0·88)	9·40 (±1·15)	N.S.

*Derived from patients' diaries.
N.S.=Not significant.

TABLE IV—DISABILITY SCORES IN 19 GROUP-2 PATIENTS

Disability scale	Mean score (±S.E.)		Significance P
	(−)-Deprenyl	Placebo	
North Western University	37·68 (±1·23)	36·79 (±0·91)	N.S.
Columbia University	22·89 (±1·61)	24·58 (±1·47)	N.S.

N.S.=Not significant.

TABLE V—FREQUENCY OF SIDE-EFFECTS

Side-effect	No. of patients
Dyskinesias	14
Nausea	9
Dry mouth	6
Dizziness	3
Postural hypotension	2
Syncope	1
Circumoral paræsthesiæ	1
Hallucinations	1
Unpleasant taste	1

self-rating depression scale. No statistically significant improvement occurred in these patients during (−)-deprenyl therapy. Although some patients described increased energy and wellbeing, in no case did this occur without concomitant physical improvement.

In doses up to 15 mg daily, (−)-deprenyl had no antiparkinsonian effects in the 5 previously untreated patients. However, a mean sparing of 200 mg levodopa daily occurred when the combined therapy was used.

Side-effects

The type and frequency of side-effects is shown in table V. All were reversible by (−)-deprenyl reduction. Dyskinesias were severe and disabling in 4 patients. No evidence of hepatotoxicity occurred after 6 months' continuous therapy. Patients took normal diets throughout the trial, and no "cheese effects" were detected.

Monoamine-oxidase Inhibition

The results of the platelet-M.A.O. determinations show that 3–5 mg (−)-deprenyl daily produce 90–100% enzyme inhibition and that after 10 mg, M.A.O. activity ceases. Some patients reported benefit only when the dose of (−)-deprenyl was increased from 5 to 10 mg daily, although greater increases rarely produced further improvement.

Discussion

Birkmayer et al.[10] thought that many of the long-term side-effects of levodopa could be avoided with smaller total daily doses, and their policy is to maintain submaximum doses of levodopa plus benserazide from the outset.[13] They have recently reported a frequency of on-off effects as low as 2·9%[13] after 5 years' levodopa treatment,[13] which compares favourably with the 20–50%[14] noted by others, including ourselves. There was no significant improvement in akinesia in the 19 patients in group 2, and side-effects were far more common than in Birkmayer's patients. This apparent discrepancy might well be explained by the fact that all our patients were receiving maximum tolerated doses of levodopa.

The precise cause of the on-off effect is unknown, but both peripheral and central biochemical changes and structural alterations are probably involved. Precise definition is also contentious: some authorities limit the term to sudden "hypotonic freezing", whereas others use it to embrace all patterns of fluctuating response in levodopa-treated patients. The frequency and nature of the various patterns of on-off effect in our patients correspond closely to those described in a recent review.[15] Dyskinesias, as well as akinesia, contributed greatly to disability. In common with other parkinsonian features, emotion and fatigue influence severity, and hospital assessment often underestimates the true extent of the disability. In general, we accepted the evidence of the patients' diaries as providing the more reliable assessment.

The tendency for patients to require 10 mg rather than 5 mg seems to indicate that whereas the lower dose is enough to cause nearly complete inhibition of platelet-M.A.O., 10 mg is required for significant brain inhibition.[9] (−)-Deprenyl, in contrast to (+)-deprenyl, which has strong amphetamine-like actions,[16] appears to exert its effects on neural transmission largely through M.A.O. inhibition. In normal volunteers, platelet-M.A.O. activity was still undetectable a day after a 10 mg dose of deprenyl: in parallel with this finding, alternate-day treatment was effective in most patients who responded well to (−)-deprenyl. Although it seems to be slightly less sensitive, brain-M.A.O. inhibition after (−)-deprenyl probably parallels inhibition of platelet-M.A.O.

In this study, (−)-deprenyl relieved only mild on-off disabilities. Some on-off effects have been attributed to levodopa toxicity,[11] and gradual reduction of dosage may relieve them.[11] These observations may explain the difference in our results and those found by Birkmayer et al.[10] in their patients with milder and less frequent on-off effects. (−)-Deprenyl in combination with levodopa seemed to act as a sustained-release preparation and was particularly beneficial for such clinical features of the disease as difficulty in turning in bed at night and early-morning immobility and stiffness.

(−)-Deprenyl has been said to be effective in potentiating the effect of levodopa only when it is combined with a peripheral dopa-decarboxylase inhibitor.[17] However, our results show no statistically significant difference in response when levodopa was given alone and when it was combined with a decarboxylase inhibitor. Nor was any significant rise in blood-pressure observed, as might have been predicted if the peripheral degradation of dopamine had been substantially prevented.[18] Presumably sufficient concentrations of M.A.O.A possessing dopamine-oxidising ability (e.g., in the gut[9]) remain to counter any excessive build-up of the circulating amine.

(−)-Deprenyl seems to have a somewhat limited role in the management of parkinsonian patients taking maximum tolerated doses of levodopa plus a peripheral dopa-decarboxylase inhibitor, but it is of considerable value for patients with end-of-dose akinesia. (−)-Deprenyl combined with smaller total daily doses of levodopa may prove useful in reducing the frequency and severity of some types of on-off effect while producing an overall benefit comparable to that observed with larger doses of levodopa.

We are grateful to Prof. J. Knoll and to the Chinoin Chemical Co., Budapest, Hungary, for generous supplies of (−)-deprenyl and to Miss

Marion Phillips for help in psychological testing. J. D. E. and A. J. L. are supported by grants from the Parkinson's Disease Society.

Requests for reprints should be addressed to G. M. S.

REFERENCES

1. Birkmayer, W., Hornykiewicz, O. *Arch. Psych. ges. Neurol.* 1962, **203**, 560.
2. Gerstenbrand, F., Prosenz, P. *Praxis*, 1965, **46**, 1373.
3. Barbeau, A., Murphy, G. F., Sourkes, T. L. *in* Monoamines et système nerveux centrale (edited by J. de Akjuriaguerra); p. 247. Geneva, 1961.
4. Hunter, K. R., Boakes, A. J., Laurence, D. R., Stern, G. M. *Br. med. J.* 1970, iii, 388.
5. Johnston, J. P. *Biochem. Pharmac.* 1968, **17**, 1285.
6. Knoll, J., Magyar, K. *in* Monoamine Oxidases—New Vistas (edited by E. Costa and M. Sandler); p. 393. New York, 1972.
7. Glover, V., Sandler, M., Owen, F., Riley, G. J. *Nature*, 1977, **265**, 80.
8. Waldmeier, P. C., Delini-Stula, A., Naître, L. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* 1976, **292**, 9.
9. Elsworth, J. D., Glover, V., Reynolds, G. D., Sandler, M., Lees, A. J., Phuapradit, P., Shaw, K. M., Stern, G. M., Kumar, P. Unpublished.
10. Birkmayer, W., Riederer, P., Ambrozi, L., Youdim, M. B. H. *Lancet*, 1977, i, 439.
11. Barbeau, A. Advances in Neurology (edited by F. McDowell and A. Barbeau); vol. 5, p. 347. New York, 1974.
12. Glover, V., Sandler, M., Grant, E., Rose, F. C., Orton, D., Wilkinson, M., Stevens, P. D. *Lancet*, 1977, i, 391.
13. Birkmayer, W. *in* Advances in Parkinsonism, (edited by W. Birkmayer and O. Hornykiewicz); p. 407. Basle, 1976.
14. Sweet, R. D., McDowell, F. H. *Ann. intern. Med.* 1975, **83**, 456.
15. Marsden, C. D., Parkes, J. D. *Lancet*, 1976, i, 292.
16. Knoll, J. *in* Monoamine Oxidase and its Inhibition (edited by G. E. W. Wolstenholme and J. Knight); p. 135. Amsterdam, 1976.
17. Birkmayer, W., Riederer, P., Youdim, M. B. H., Linauer, W. *J. neural Transm.* 1975, **36**, 303.
18. Goldberg, L. I. *Pharmac. Rev.* 1972, **24**, 1.

DEPRENYL IS METABOLIZED TO METHAMPHETAMINE AND AMPHETAMINE IN MAN

(—)-Deprenyl (Figure 1) is a monoamine oxidase (MAO) inhibitor with selective action against the B form of the enzyme (Knoll, 1976). Its administration prevents the degradation of dopamine in human brain, where this is a substrate for MAO B (Glover, Sandler, Owen & Riley, 1977); however, it leaves the peripheral mechanisms normally preventing a hypertensive response following tyramine administration intact (Elsworth, Glover, Reynolds, Sandler, Lees, Phuapradit, Shaw, Stern & Kumar, 1978), although this amine interacts adversely with all other irreversible MAO inhibitors so far described. Because of this freedom from what has come to be called the 'cheese effect', deprenyl, in combination with L-dopa, provides both a rational and safe therapy for the treatment of Parkinson's disease (Birkmayer, Riederer, Ambrozi & Youdim, 1977; Lees, Shaw, Kohout, Stern, Elsworth, Sandler & Youdim, 1977).

Little is known of the metabolism of deprenyl either in man or animals. However, by analogy with that of the not dissimilar MAO inhibitor, pargyline (N-methyl-N-propynylbenzylamine), which is metabolized in mammals to benzylamine (Edwards & Blau, 1973; Durden, Philips & Boulton, 1976), it seemed possible that deprenyl might be degraded to amphetamine. Indeed, a preliminary *in vitro* study (unpublished) showed that rat liver homogenates convert some of the drug to amphetamine and methamphetamine. We therefore sought to identify these compounds in human urine after the administration of therapeutically-active doses of (—)-deprenyl.

Urine samples (24 h) were collected from six normal male volunteers on the third day each of test and placebo administration during the course of a double-blind crossover study of the effects of (—)-deprenyl hydrochloride on sleep. The volunteers, who received either 5 or 10 mg of this drug (Table 1) in a single dose daily, were free from other medication whilst the experiment lasted.

Urine was stored at —20°C and thawed immediately before assay. Amphetamines in 0.5 ml urine were quantified after adding an internal standard, *p*-methylphenylethylamine. Samples were then subjected to acid hydrolysis (pH 1) at 100°C for 1 h to release any conjugates present. Quantification was achieved by extraction of the amines from alkaline urine into hexane, back-extraction into acid, derivatization with pentafluorobenzoyl chloride and

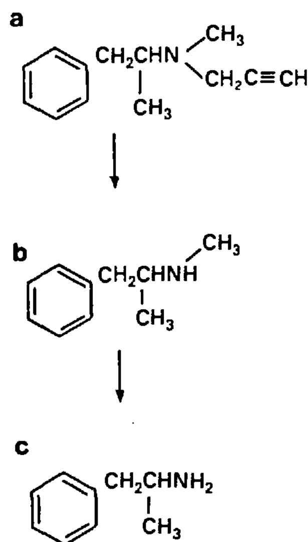


Figure 1 The structural formulae of a) deprenyl, b) methamphetamine and c) amphetamine.

gas chromatography-mass spectrometry (GC-MS) of the resultant pentafluorobenzamides (Reynolds, King, Elsworth & Sandler, unpublished observations). This method of detection involves monitoring ions of m/e 118 (corresponding to methyl-substituted phenylethyl moieties) and is specific for the two amphetamines and the internal standard used here.

Unchanged deprenyl was sought by extracting alkaline urine with ether, back-extracting into acid and re-extracting into ether after adding excess alkali. For quantification by GC-MS, this ether solution was injected directly on to a 6 ft column of PPE-21 at 200°C, monitoring the major ions associated with the mass spectrum of the drug (m/e 56 and 96).

Urinary output values of the two major metabolites of deprenyl are shown in Table 1. Substantial amounts of methamphetamine and rather less amphetamine were identified in all samples collected during drug administration but were not present in control specimens. Methamphetamine accounted for a quantitatively major proportion of the administered dose, with rather less amphetamine being excreted. The simple relationship between (—)-deprenyl and these two compounds is shown in Figure 1. It is of interest that methamphetamine output was greatest in the two most highly acid urine samples. Although this finding may have occurred by chance, it is consistent with the known effect of urinary pH on the excretion of this compound (Beckett & Rowland, 1965). Indeed, the anomalously high excretion value in subject 2 might be explicable in terms of an alkaline urine on the previous day causing relative methamphetamine retention, with later rebound.

The proportion of this amine excreted after deprenyl ingestion is comparable with that detected after an oral dose of methamphetamine itself (Beckett & Rowland, 1965). Amphetamine is probably derived from the demethylation of methamphetamine (Figure 1), although demethylation of (—)-deprenyl followed by loss of the propynyl group cannot be ruled out. Thus virtually all administered (—)-deprenyl was metabolized via methamphetamine by an as yet unknown enzymatic mechanism. A careful search for unmetabolized deprenyl proved negative, even though the method employed is sensitive down to a concentration of less than 10 ng/ml, corresponding to no more than 1% of the administered dose.

The dealkylation process seems likely to take place in the liver (Caldwell, 1976) and, because of their lipophilic nature, the amphetamines liberated into the blood stream are likely to cross the blood-brain barrier to gain access to the brain. Thus, corresponding pharmacological effects would presumably be evident if the dose were sufficiently large. It is here that an important point must be taken into account: the (—)-isomer of deprenyl possesses substantially greater MAO inhibitory action than the (+)-form (Knoll, 1976) and the former alone is now used in clinical practice. However, the central effect of (+)-amphetamine is 3–4 times larger than that of the (—)-isomer (Innes & Nickerson, 1977). Although we have not yet made direct measurements of the optical activity of the amphetamines generated from deprenyl, it seems likely that they will show the (—)-configuration, for Magyar, Vizi, Ecsery & Knoll (1967) showed that (+)-deprenyl has considerably greater amphetamine-like action than its (—)-isomer.

What is the significance of these findings in clinical practice? The therapeutically-effective dose of (—)-deprenyl given in combination with L-dopa in parkinsonism is no more than 10–15 mg (Birkmayer *et al.*, 1977; Lees *et al.*, 1977), an amount unlikely to produce any marked degree of central amphetamine-like action, although a contribution of amphetamine to the total pharmacological response cannot be ruled out. Indeed, some improvement in disability was demonstrated during a recent trial of (+)- and (—)-amphetamine in Parkinson's disease (Parkes, Tarsy, Marsden, Bovill, Phipps, Rose & Asselman, 1975); those patients on L-dopa plus amphetamine derived greater benefit than those on L-dopa or on amphetamine alone. Perhaps the benefit accruing from the L-dopa plus deprenyl treatment combination should be reassessed in the light of these considerations.

The unique lack of 'cheese effect' (Elsworth *et al.*,

Table 1 Urinary output of amphetamine and methamphetamine, the two major metabolites of deprenyl

Subject	(—)-Deprenyl HCl (mg/24 h)	Urine volume (ml)	pH	Amphetamine excretion (mg)	Amphetamine excretion Equivalent % dose	Methamphetamine excretion (mg)	Methamphetamine excretion Equivalent % dose
1	5	1435	6.4	0.48	15.8	1.67	50.2
2	5	1400	5.9	0.59	19.5	3.47	104.0
3	10	2100	6.1	1.05	17.3	3.42	51.3
4	10	1190	6.4	0.88	14.5	3.55	53.2
5	10	1280	5.8	0.67	11.1	4.98	74.6
6	10	1600	6.8	0.74	12.2	3.08	46.2
Mean					15.1		63.3

1978) of this MAO inhibitor might even be explicable in terms of the protection by chronic amphetamine treatment on the tyramine pressor response in man (Cavanaugh, Griffith & Oates, 1970). Freedom from this effect is still apparent at substantially higher deprenyl dosage (Stern, Lees & Sandler, 1978). It seems possible that such regimens will be helpful in depressive illness, if preliminary trials can be confirmed (Varga & Tringer, 1967; Mendlewicz & Youdim, 1978). If subsequent investigations show high dosage (–)-deprenyl treatment to be useful in depression, it may well be necessary to minimize the risk of adverse reactions, e.g. amphetamine psychosis (Kety, 1959), by maintaining a maximally acid urine to facilitate amphetamine excretion (Beckett & Rowland, 1965).

G.P.R. is supported by the Schizophrenia Research Fund and J.D.E. and A.J.L. by the Parkinson's Disease Society. We thank Dr G.S. King and Mr B.R. Pettit for mass spectrometric assistance. We are grateful to Prof. J. Knoll and to the Chinoin Co., Budapest, for generous supplies of deprenyl.

G.P. REYNOLDS, J.D. ELSWORTH, K. BLAU, M. SANDLER, A.J. LEES[1] & G.M. STERN[1]

Bernhard Baron Memorial Research Laboratories and Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG

and

[1] *Department of Neurology, University College Hospital, Gower Street, London WC1E 6AU*

Received August 14, 1978

Note added in proof:

The recent paper by Simpson (1978) shows (–)-deprenyl to possess indirectly acting sympathomimetic properties *in vivo*. We wish to point out that these findings are explicable in terms of the pharmacological effects of the metabolites of (–)-deprenyl.

References

BECKETT, A.H. & ROWLAND, M. (1965). Urinary excretion of methylamphetamine in man. *Nature, Lond.*, **206**, 1260–1261.

BIRKMAYER, W., REIDERER, P., AMBROZI, L. & YOUDIM, M.B.H. (1977). Implications of combined treatment with 'Madopar' and L-deprenyl in Parkinson's disease. *Lancet*, **i**, 439–443.

CALDWELL, J. (1976). The metabolism of amphetamines in mammals. *Drug Metab. Revs.*, **5**, 219–280.

CAVANAUGH, J.H., GRIFFITH, J.D. & OATES, J.A. (1970). Effect of amphetamine on the pressor response to tyramine: Formation of *p*-hydroxynorephedrine from amphetamine in man. *Clin. Pharmac. Ther.*, **11**, 656–664.

DURDEN, D.A., PHILIPS, S.R. & BOULTON, A.A. (1976). Identification and distribution of benzylamine in tissue extracts isolated from rats pretreated with pargyline. *Biochem. Pharmac.*, **25**, 858–859.

EDWARDS, D.J. & BLAU, K. (1973). Phenylethylamines in brain and liver of rats with experimentally induced phenylketonuria-like characteristics. *Biochem. J.*, **132**, 95–100.

ELSWORTH, J.D., GLOVER, V., REYNOLDS, G.P., SANDLER, M., LEES, A.J., PHUAPRADIT, P., SHAW, K.M., STERN, G.M. & KUMAR, P. (1978). Deprenyl administration in man: a selective monoamine oxidase B inhibitor without the 'cheese effect'. *Psychopharmacology*, **57**, 33–38.

GLOVER, V., SANDLER, M., OWEN, F. & RILEY, G.J. (1977). Dopamine is a monoamine oxidase B substrate in man. *Nature, Lond.*, **265**, 80–81.

INNES, I.R. & NICKERSON, M. (1977). Norepinephrine, epinephrine and the sympathomimetic amines. In: *The Pharmacological Basis of Therapeutics*. ed. Goodman, L.S. & Gilman, A. pp. 477–513. New York: Macmillan.

KETY, S.S. (1959). Biochemical theories of schizophrenia. *Science*, **129**, 1528–1532.

KNOLL, J. (1976). Analysis of the pharmacological effects of selective monoamine oxidase inhibitors. In *Monoamine Oxidase and its Inhibition*, ed. Wolstenholme, G.E.W. & Knight, J. pp. 135–161, Amsterdam: Elsevier.

LEES, A.J., SHAW, K.M., KOHOUT, L.J., STERN, G.M., ELSWORTH, J.D., SANDLER, M. & YOUDIM, M.B.H. (1977). Deprenyl in Parkinson's disease. *Lancet*, **ii**, 791–796.

MAGYAR, K., VIZI, E.S., ECSERY, Z. & KNOLL, J. (1967). Comparative pharmacological analysis of the optical isomers of phenyl-isopropylmethyl-propinylamine (E-250). *Acta Physiol. Acad. Sci. Hung.*, **32**, 377–387.

MENDLEWICZ, J. & YOUDIM, M.B.H. (1978). The potentiation of the antidepressant properties of 5-hydroxytryptophan by deprenil. *J. Neural Transmiss.* (in press).

PARKES, J.D., TARSY, D., MARSDEN, C.D., BOVILL, K.T., PHIPPS, J.A., ROSE, P. & ASSELMAN, P. (1975). Amphetamines in the treatment of Parkinson's disease. *J. Neurol. Neurosurg. Psychiat.*, **38**, 232–237.

SIMPSON, L.L. (1978). Evidence that deprenyl, a type B monoamine oxidase inhibitor, is an indirectly acting sympathomimetic amine. *Biochem. Pharmac.*, **27**, 1591–1595.

STERN, G.M., LEES, A.J. & SANDLER, M. (1978). Deprenyl in Parkinson's disease. *J. Neural Transmiss.* in press.

VARGA, E. & TRINGER, L. (1967). Clinical trial of a new type of promptly acting psychoenergetic agent (phenyl-isopropylmethyl-propinylamine-HCl, E-250). *Acta Med. Acad. Sci. Hung.*, **23**, 289–295.

Catecholamines: Basic and Clinical Frontiers (1979) Eds. Usdin, E., Kopin, I.J., Barchas, J. Proceedings of the Fourth Catecholamine Symposium, Asilomar, California. Pergamon, New York.

SELECTIVE MAO INHIBITION: A NEW STRATEGY IN THE TREATMENT OF PARKINSON'S DISEASE

J.D.Elsworth, V.Glover and M.Sandler
Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London W6 OXG, U.K.

ABSTRACT

Although conventional monoamine oxidase (MAO) inhibitors ought to be useful in the L-dopa treatment of parkinsonism, the combination causes a hypertensive reaction, a variant of the tyramine-induced "cheese effect." We have shown deprenyl, a selective and clinically effective MAO B inhibitor, to be free from such adverse reactions in volunteers. Its mechanism of action is not clear cut.

Deprenyl is an inhibitor of the so-called B form of MAO (1). Substrates for this type of MAO include phenylethylamine (PE) (2) and dopamine (DA) in human brain (3). (-)-Deprenyl combined with L-dopa therapy has been used in the treatment of Parkinson's disease and its role has now been assessed by several groups. The benefit seems to be restricted to the relief of certain types of "on-off" effect (4,5). MAO inhibitors were used previously to potentiate the antiparkinsonian actions of L-dopa (6), but the combination had to be abandoned because hypertensive crises were provoked (7). This problem can also occur when certain drugs and foodstuffs, especially cheese, which contains tyramine are ingested by patients undergoing MAO inhibitor therapy (8). However, deprenyl may be free from this "cheese effect" (1). We investigated this claim with volunteers, both untreated normal and parkinsonian subjects taking L-dopa with or without a decarboxylase inhibitor, by gauging their tolerance to oral tyramine before and after one week of deprenyl administration (9).

After this treatment, all subjects were still able to consume amounts of tyramine far larger than those encountered in a normal diet. MAO B inhibition by deprenyl was confirmed by assaying platelet MAO activity, and by measuring elevated concentrations of PE in the urine. However the urine of parkinsonian patients taking carbidopa in addition to L-dopa and deprenyl (10 mg) did not show a rise in concentration of PE, whereas those patients receiving L-dopa with deprenyl or deprenyl alone had an elevated output of the amine. Thus carbidopa seems to be preventing increased excretion of PE, possibly by inhibiting the decarboxylation of phenylalanine. Presumably though this would be a peripheral effect. In fact, raised levels of PE have also been found in post-mortem brains from parkinsonians who had been taking deprenyl with a decarboxylase inhibitor (10). This build-up of PE could conceivably play a part in the therapeutic response of deprenyl in its own right or could enhance the effects of DA by competing with it for uninhibited MAO (11).

The failure of deprenyl to elicit a "cheese effect" may perhaps be explained in terms of selective inhibition of MAO. Tyramine is a substrate for the A and B forms of MAO and we have shown (9) that human intestinal mucosa, which possesses type A activity only (12), is relatively resistant to deprenyl inhibition, and so presumably would continue to inactivate tyramine and prevent dangerous amounts entering the circulation. This interpretation fits the observation that clorgyline, a MAO A inhibitor, strongly potentiates the tyramine pressor response (13). Even so, deprenyl only exerts its selectivity within a certain concentration range (9). When deprenyl was repeatedly administered to rats a gradual decrease in specificity of inhibition towards brain and liver MAO was noted (14). From these facts, one might have anticipated that protection from the "cheese effect" would be a transient phenomenon. However, parkinsonian patients who had received deprenyl daily for as long as a year showed no real alteration in their tolerance to tyramine (15). The finding of Egashira et al (16) may be relevant here; they showed that whereas increasing the incubation time of deprenyl with rat liver mitochondria results in decreased selectivity, the mode of inhibition towards 5HT is, in fact, reversible. Knoll (12) has shown that deprenyl, albeit in high doses, prevented tyramine potentiating certain noradrenergic responses in cats, and concluded it was halting uptake of tyramine and release of noradrenaline. That such mechanisms occur to prevent the cheese reaction recently received support from Sandler et al (18). In this instance the pig was chosen as experimental animal because apart from MAO A in its gut wall, tyramine in this species is degraded by the B variety of the enzyme (12). If freedom from the cheese reaction in man relies on preserving the gastrointestinal MAO A barrier, then by-passing this barrier by intravenous tyramine administration after deprenyl treatment should have elicited a hypertensive response. However, despite considerable inhibiton of the enzyme, no such reaction occurred. In contrast, a clorgyline treated group of pigs suffered a profound pressor response to tyramine, even though MAO was only inhibited to a minor degree. Thus, the ability of a drug to cause a "cheese effect" may well be dissociated from its MAO inhibitory properties. It may well be safe to employ deprenyl in doses where it inhibits both A and B forms. The drug may even, conceivably, afford protection against the "cheese effect" if co-administered with another MAO inhibitor.

At least part of the therapeutic response, together with yet another possible explanation for freedom from the "cheese effect," may derive from the metabolic products of deprenyl. We have found it to be largely converted to methamphetamine and amphetamine (19). It has long been recognised that amphetamine has an anti-parkinsonian action (20), and a diminished pressor response to tyramine has been described after chronic amphetamine treatment (21). Although it seems likely that the less centrally active (-)-isomers are generated (19) there may be a risk of dependence inherent in deprenyl therapy, although as yet this has not been reported.

ACKNOWLEDGEMENTS

We are grateful to Professor J.Knoll and the Chinoin Chemical Company, Budapest for generous supplies of deprenyl. J.D.E. is supported by a grant from the Parkinson's Disease Society, and V.G. by a grant from the Migraine Trust.

REFERENCES

1. J.Knoll, Analysis of the pharmacological effects of selective monoamine oxidase inhibitors, in Monoamine Oxidase and its Inhibition, eds.G.E.W. Wolstenholme and J.Knight, Elsevier, Amsterdam, pp.135-161 (1976).

2. H.-Y.T.Yang and N.H.Neff, β-phenylethylamine: a specific substrate for type B monoamine oxidase of brain, J.Pharmac.exp.Ther. 187, 365-371 (1973).

3. V.Glover,M.Sandler,F.Owen,G.F.Riley, Dopamine is a monoamine oxidase B substrate in man, Nature, 265, 80-81 (1977).

4. W Birkmayer,P.Riederer,M.B.H.Youdim and W.Linauer, The potentiation of the anti-akinetic effect after L-dopa treatment by an inhibitor of MAO B, Deprenil, J.Neural Transmiss. 36, 303-326 (1975).

5. A.J.Lees,K.M.Shaw,L.J.Kohout,G.M.Stern,J.D.Elsworth,M.Sandler and M.B.H.Youdim, Deprenyl in Parkinson's disease, Lancet ii, 791-795 (1977).

6. W.Birkmayer and O.Hornykiewicz, Der L-Dioxyphenylalanin (=Dopa)-Effekt beim Parkinson-Syndrom des Menschen, Arch.Psych.Nervenkr.203, 560-571 (1962).

7. K.R.Hunter,A.J.Boakes,D.R.Laurence and G.M.Stern, Monoamine oxidase inhibitors and L-dopa, Brit.med.J. 3, 388 (1970).

8. E.Marley and B.Blackwell, Interactions of monoamine oxidase inhibitors, amines and foodstuffs, Advanc.Pharmac.Chemother. 8, 186-239 (1970).

9. J.D.Elsworth,V.Glover,G.P.Reynolds,M.Sandler,A.J.Lees,P.Phuapradit,K.M.Shaw, G.M.Stern and P.Kumar, Deprenyl administration in man: a selective monoamine oxidase B inhibitor without the "cheese effect." Psychopharmacology 57, 33-38 (1978).

10. G.P.Reynolds,P.Riederer,M.Sandler,K.Jellinger and D.Seeman, Amphetamine and 2-phenylethylamine in post-mortem parkknsonian brain after deprenyl administration, J.Neural Transmiss. in press.

11. H.L.White and J.C.Wu, Multiple binding sites of human brain monoamine oxidase as indicated by substrate competition, J.Neurochem. 25, 21-26 (1975).

12. R.F.Squires, Multiple forms of monoamine oxidase in intact mitochondria as characterised by selective inhibitors and thermal stability: a comparison of eight mammalian species, in Monoamine Oxidases - New Vistas, Adv.Biochem. Psychopharmacol.5, eds.E.Costa and M.Sandler, Raven, New York,pp.355-370 (1972).

13. M.H.Lader,G.Sakalis and M.Tansella, Interactions between sympathomimetic amines and a new monoamine oxidase inhibitor, Psychopharmacologia 18, 118-123 (1970).

14. P.C.Waldmeier and A.E.Felner, Deprenil: loss of selectivity for inhibition of B-type MAO after repeated treatment, Biochem.Pharmacol. 27, 801-802 (1978).

15. G.M.Stern,A.J.Lees and M.Sandler, J.Neural Transmiss, in press.

16. T.Egashira,B.Ekstedt and L.Oreland, Inhibition by clorgyline and deprenyl of the different forms of monoamine oxidase in rat liver mitochondria, Biochem.Pharmacol, 25, 2583-2586 (1976).

17. J.Knoll and K.Magyar,Some puzzling pharmacological effects of monoamine oxidase inhibitors, in Monoamine Oxidase - New Vistas, Adv.Biochem.Psychopharmacol.5, eds.E.Costa and M.Sandler, Raven,New York,pp.393-408 (1972).

18. M.Sandler,V.Glover,A.Ashford and G.M.Stern, Absence of "cheese effect" during deprenyl therapy. Some recent studies, J.Neural Transmiss. in press.

19. G.P.Reynolds,J.D.Elsworth,K.Blau and M.Sandler, Brit.J.Clin.Pharm. in press.

20. I.Finkelman and L.B.Shapiro, Benzedrine sulfate and atropine in treatment of chronic encephalitis, J.Amer.Med.Ass. 109, 344-346 (1937).

21. J.H.Cavanaugh,J.D.Griffith and J.A.Oates, Effect of amphetamine on the pressor response to tyramine: Formation of p-hydroxynorephedrine from amphetamine in man, Clin.Pharm.Therap. 11, 656-663 (1970).

Catecholamines: Basic and Clinical Frontiers (1979) Eds. Usdin, E., Kopin, I.J., Barchas, J. Proceedings of the Fourth Catecholamine Symposium, Asilomar, California. Pergamon, New York.

DOPAMINE OXIDATION AND ITS INHIBITION BY (-)-DEPRENYL IN HUMAN BRAIN AND TISSUES

Vivette Glover, J.D.Elsworth and M. Sandler
Bernhard Baron Memorial Research Laboratories, Queen Charlotte's Maternity Hospital, London W6 OXG, U.K.

ABSTRACT

Dopamine (DA) is predominantly metabolised by (-)-deprenyl sensitive monoamine oxidase (MAO) B in the DA rich regions of the human brain. In other tissues a larger part is also metabolised by MAO A.

INTRODUCTION

(-)-Deprenyl, a selective MAO B inhibitor without the "cheese effect" (1), is being used in the treatment of Parkinson's disease, (2) (3), and in trials as an antidepressant. Relating the effects of selective doses of the drug in man to its biochemical action, may help to clarify the role of different amines and of MAO B in certain normal and pathological states. Present evidence suggests that MAO A is the major route for the oxidation of noradrenaline and 5-hydroxytryptamine (5-HT)(4), and DA (5) in the rat. In the human striatum, however, DA is predominantly oxidised by MAO B(6). In this study we have used (-)-deprenyl as a tool for in vitro examination of the nature of DA oxidation in different human brain regions and other tissues. The specific substrates, 5-HT for MAO A, and phenylethylamine (PEA) for MAO B, are used to examine the A/B activity ratio in different preparations.

METHODS

MAO was assayed radiometrically (1). Human tissues and brains were stored at 5°C for 12-36 hours post-mortem, then at -20°C until assay. Brains were frozen in a mould and just before dissection, allowed to warm to about -10°C, then cut into 2 - 3 mm slices. 10% w/v homogenates were prepared in 50mM phosphate buffer and assayed in duplicate. Jejunal tissue was obtained from biopsy material (1).

RESULTS

The inhibition of 5-HT and PEA oxidation by different concentrations of (-)-deprenyl was determined. Human cortex and caudate samples gave similar patterns, although some variation was found with different samples of each. In all samples 10^{-6}M was the most selective concentration, MAO B being 80 - 100% inhibited and MAO A 0 - 20%. We have, therefore, used 10^{-6}M deprenyl to distinguish whether DA is behaving as an A or B substrate in different preparations.

TABLE 1 Distribution of 5HT, PEA and DA oxidising activities in human tissues, and inhibition of DA oxidation by 10^{-6}M (-)-deprenyl

Brain	5HT	PEA	DA	5HT/PEA	% inhibition of DA by deprenyl
Accumbens	21.5	20.5	27.0	1.0	85
Caudate	21.0	16.6	30.7	1.3	82
Pallidus	20.1	15.3	24.7	1.3	83
Putamen	15.9	11.5	18.7	1.3	81
Hypothalamus	39.0	20.4	36.3	1.9	73
Amygdala	31.2	14.2	25.6	2.2	76
Precentral cortex	18.1	6.9	14.0	2.6	67
Occipital cortex	21.0	5.8	17.8	3.6	70
Temporal cortex	18.6	7.7	14.3	2.4	66
Frontal cortex	17.4	6.6	12.3	2.6	70
Cerebral cortex	12.8	5.5	11.3	2.3	62
Tissues					
Heart	4.0	5.9	9.1	0.7	85
Lung	9.4	3.8	9.2	2.4	22
Kidney	31.6	14.5	27.5	2.1	64
Liver	52.0	23.2	39.7	2.2	60
Jejunum	55.9	7.8	62.4	7.1	34
Platelet	<2	6.3	14.9	<0.3	48

Activities are expressed as nmoles substrate oxidised /mg protein/30min. Results are the means of duplicate assays with samples from at least 4 individuals.

Table 1 shows the distribution of the 5-HT, PEA and DA oxidising activities in different regions of the human brain. In all the brains studied the B/A ratio was highest in the DA rich regions of the striatum and nucleus accumbens. The inhibition of DA oxidation by 10^{-6}M (-)-deprenyl showed that in these regions DA was largely metabolised by MAO B. However, DA was not exclusively metabolised by a deprenyl sensitive component, and in the cortical regions a higher proportion was deprenyl resistant.

Table 1 also shows the results of a similar analysis of some other human tissues. DA oxidation can clearly be associated with MAO A as shown in the jejenum and with MAO B as in the platelet. In the kidney and liver there was substantial activity with all three substrates and DA oxidation was partially sensitive to selective (-)-deprenyl inhibition. The heart had a high B/A ratio and in 3 of the 5 individuals examined there was almost no MAO A activity and DA oxidation was entirely inhibited by 10^{-6}M (-)-deprenyl.

DISCUSSION

These results show that 10^{-6}M (-)-deprenyl can largely inhibit DA oxidation in the DA rich regions of the human brain leaving 5-HT oxidation unimpaired. A dose of 10mg distributed in a body water mass of 40kg would give approximately this concentration. However, it may be that a smaller dose is needed to maintain selectivity with chronic administration.

These results provide no evidence for a specific MAO for DA. There is no tissue or region of the human brain where the level of DA oxidising activity or its response to (-)-deprenyl cannot be explained by the additive results of its being a substrate for the MAO A and B present. As MAO A can metabolise noradrenaline, 5-HT, and DA, the physiological reason for the existence of an MAO B remains an unanswered question. Is its function partly to metabolise a trace amine such as PEA, methylhistamine, or some other unknown amine?

ACKNOWLEDGEMENTS

We are grateful to Professor J. Knoll and the Chinoin Chemical Company, Budapest for generous supplies of deprenyl, J.D.E. is supported by a grant from the Parkinson's Disease Society, and V.G. by a grant from the Migraine Trust.

REFERENCES

1. J.D. Elsworth, V. Glover, G.P. Reynolds, M. Sandler, A.J. Lees, P. Phuapradit, K.M. Shaw, G.M. Stern and P. Kumar, Deprenyl administration in man: a selective monoamine oxidase B inhibitor without the "cheese effect". Psychopharmacology 57, 33-38 (1978).

2. A.J. Lees, K.M. Shaw, L.K. Kohout, G.M. Stern, J.D. Elsworth, M. Sandler and M.B.H. Youdim, Deprenyl in Parkinson's disease Lancet ii 791-795 (1977).

3. W. Birkmayer, P. Riederer, and L. Ambrozi, Implication of combined treatment with "Madopar" and L-Deprenil in Parkinson's Disease. A long term study. Lancet i 439-443 (1977).

4. K.F. Tipton, M.D. Houslay and T.J. Mantle, in Monoamine Oxidase and its Inhibition pp 5-15 Ciba Foundation Symposium 39, Amsterdam, Ellsevier-Excerpta Medica - North Holland (1976).

5. P.C. Waldmeier, A. Delini-Stula, and L. Maître, Preferential deamination of dopamine by an A type monoamine oxidase in rat brain, Naunyn-Schmiedeberg's Arch Pharmac 292 9-14 (1976).

6. V. Glover, M. Sandler, F. Owen and G.J. Riley, Dopamine is a monoamine oxidase B substrate in man, Nature 265 80-81 (1977).