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NOVEL (−)DEPRENYL-DERIVED SELECTIVE INHIBITORS OF B-TYPE MONOAMINE OXIDASE. THE RELATION OF STRUCTURE TO THEIR ACTION

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Abstract—A structure–activity relationship study has revealed the importance of the aromatic ring and the optimum length of the chain between the aromatic ring and the nitrogen for the selective inhibition of MAO-B. Among the (−)deprenyl derived new compounds described in this study *N*-methyl-*N*-propargyl-(2-furyl-l-methyl)-ethylammonium. HCl(U-1424) seems to be the most promising selective inhibitor of MAO-B.

Many authors working with reversible inhibitors (e.g. harmala alkaloids) observed that a monoamine oxidase (MAO) inhibitor may block the oxidation of some amines more than that of others [1]. A new approach to MAO and its inhibition started, however, with the discovery of the selectivity of two highly potent, irreversible inhibitors, deprenyl and clorgyline.

In 1964 Knoll *et al.* [2] developed deprenyl (2-phenyl-1-methyl-*N*-methyl-propinylamine. HCl, E-250) and described it as a new spectrum inhibitor of MAO [2–5]. A detailed analysis of the pharmacological and biochemical effects of this compound proved that (−)deprenyl is a highly selective inhibitor of a special type of MAO which preferentially deaminates benzylamine, metaiodobenzylamine [6], and mainly phenylethylamine (PEA) [7].

Clorgyline (2,4-dichlorophenoxypropyl-*N*-methyl-propinylamine), a compound similar in structure to deprenyl, was developed in 1968 by Johnston [8] who found it to be a selective inhibitor of that type of MAO which preferentially deaminates serotonin (5-HT). In order to distinguish the two forms of MAO (the one highly selective to clorgyline and the one which is relatively insensitive) Johnston introduced the terms "A" and "B" MAO [8]. This nomenclature has become widely accepted. MAO-A is selectively inhibited by clorgyline and MAO-B by (−)deprenyl. These two compounds are now widely used in physiological, pharmacological and biochemical studies of the two main types of mitochondrial MAO [9–23].

A comparative study with (−)deprenyl and clorgyline led to the conclusion that MAO-A is specialized for binding and metabolizing the ethylamine side chain of the substrate if it belongs to a 5-hydroxyindolamine (serotonin oxidase) and MAO-B is specialized for recognizing and metabolizing phenylethylamine (phenylethylamine oxidase). There are many other amines which, because of structural similarities, are substrates of either MAO-A or MAO-B or are common substrates of both enzymes [24] but 5-HT has the lowest K_m for MAO-A and PEA has the lowest K_m for MAO-B [25]. Studies of structure–activity relationship allowed the hypothesis that the binding of the aromatic ring, the CH_2–CH_2 chain between the aromatic ring and nitrogen moiety and the attachment of the nitrogen to the enzyme determine the affinity of the substrates for the enzyme and their ability to be metabolized on the surface of the enzyme. MAO-A and B are thought to differ from each other in the complementary parts of the aromatic ring [26,27]. In MAO-A this part of the enzyme is adapted for the attachment of the 5-hydroxyindol ring in serotonin, whereas in MAO-B it is a more simple flat surface for the binding of the benzene ring. The other sites of the two enzymes interacting with the CH_2–CH_2 chain and the nitrogen are the same [26]. The site of the enzyme for the attachment of the nitrogen was proposed to be located in vicinity of the covalently bound flavin group of MAO-A and B. This situation offers a good explanation for the destruction of MAO-A and B by their respective selective inhibitors, clorgyline and (−)deprenyl [27]. Both contain a propargyl-group attached to the nitrogen and this group is known to be capable of reacting irreversibly with the covalently bound flavin [28]. It was recently demonstrated that [^{14}C]deprenyl binds in stoichiometric fashion to the flavin active site of MAO on position 5 of riboflavin [29].

A detailed analysis of the therapeutic aspects of the selective inhibition of MAO-B is more promising with respect to the hazards of combination with a variety of foods and drugs [24, 26, 27]. Therefore we studied in more detail the chemical features associated with the selective inhibition of MAO-B and the results of this study are now presented.

MATERIALS AND METHODS

Test animals. Male CFY rats weighing 100 ± 10 g, CFLP male mice of 20 g body wt and cats of both sexes weighing 2–3.5 kg were used. The rats and mice were kept on standard laboratory diet.

Radioactive substrates. Tyramine-1[^{14}C]HBr ([^{14}C]tyramine), sp. act.: 10,1 mCi/m-mole, NENC, Boston; 5-hydroxy-2[^{14}C]tryptamine, creatinesulphate ([^{14}C]5-HT) sp. act.: 59 mCi/m-mole, Amersham, England: β-phenylethylamine-1[^{14}C]HCl

([^{14}C]PEA), sp. act.: 9.86 mCi/m-mole, NENC, Boston, MA.

Method for the preparation of mitochondria. Inhibitor treated and untreated rats were decapitated and the brain and liver were homogenized in 0.25 M sucrose solution containing 0.5 mM EDTA (pH 7.4) to give a 10% (w/v) preparation. In the *in vitro* experiments, mitochondrial fractions were prepared from the organs. The mitochondrial fraction was prepared with differential centrifugation from brain homogenate, by the method of Aldridge [30] and from liver tissue by that of Johnson [31]. The mitochondrial fraction was kept at 10°C and it had been used for at least 2 weeks. The brain and liver mitochondrial preparation contained 26.6 ± 1.9 and 38.7 ± 2.6 mg/ml of protein, respectively. Protein determination was carried out by the method of Lowry *et al.* [32].

The inhibitor was i.v. administered in 0.2 ml physiological saline, 1 hr before decapitation. For the determination of MAO activity 10% homogenates of brain and liver were used.

Radiometric determination of MAO activity. MAO activity was measured by the radiometric method of Wurtman and Axelrod [33]. The volume of the incubation mixture was 1.0 ml containing 0.01 M phosphate buffer (pH 7.4); 0.001 M EDTA (pH 7.4); ^{14}C-labelled substrate (tyramine, 5-HT or PEA), 0.1 ml mitochondrial preparation or homogenate and inhibitor of different concentration. Of the radioactive substrate, 10^5 d.p.m. per tube was used. The final concentration of tyramine and serotonin was 0.0025 M, that of PEA was 0.00025 M. Working with PEA as substrate the brain and the liver mitochondrial preparations were diluted to five and ten, the homogenate to three and five times, respectively, with the phosphate buffer.

Reaction was initiated by addition of the labelled substrates. When tyramine or serotonin were the substrates, a 10 min incubation time was used; in the case of PEA it was 30 min. Incubation was carried out in a Dubnoff metabolic shaker (Vibroterm, Labor) in carbogenic atmosphere (95% O_2 + 5% CO_2), at 37.5°, in glass stoppered tubes having 10 ml volume. The reaction was stopped by the addition of 0.2 ml 2 M citric acid. The extraction of the labelled aldehyde formed during the reaction was carried out with 5.0 ml ethylacetate and after 30 min centrifugation time (1500 *g*), 4.0 ml of the organic phase was added to 10 ml toluol scintillator for the determination of radioactivity.

In the studies *in vitro* the mitochondrial preparations was preincubated for 10 min with the inhibitor. The inhibitory potency *in vitro* of a compound was determined by using at least six different concentrations of the inhibitor (between 10^{-8} and 10^{-3} M) and the concentration of the compound which caused 50 per cent inhibition (ID_{50}) was calculated from the inhibition curve. The effect of each dose was calculated from five experiments and expressed as percentual inhibition of the control ± S.E.M. The MAO-inhibitors were i.v. injected 1 hr prior to the removal of the brain and liver.

Method for checking the effect of selective MAO inhibitors in vivo *in the cat using the nictitating membrane as the detector*

The technique introduced by Knoll [24] was used. The endogenous substrate having the highest affinity for MAO-A is 5-HT and that for MAO-B is PEA. The nictitating membrane of the cat responds to the i.v. injection of both 5-HT and PEA with dose-dependent contractions. The lowest dose which usually evokes a contraction of the nictitating membrane is 10–15 μg/kg in the case of 5-HT and 100–200 μg for PEA. On the other hand, much lower doses of the same amines are active as the liver is excluded from the circulation [26], showing that both amines are metabolized by MAO in the liver. (−)Deprenyl, the selective inhibitor of MAO-B strongly potentiates the effects of PEA on the nictitating membrane in a dose range which leaves the effect of 5-HT essentially unchanged. Therefore we used this method to follow *in vivo*, the selective MAO-B inhibitory effect of our new compounds in the cat. The experiments were carried out in cats under chloralose–urethane anaesthesia (30 and 200 mg/kg body wt, respectively) and artificial respiration. Blood clotting was prevented by 5 mg/kg heparin injected at the beginning of the experiment into the femoral vein. Contractions of the nictitating membrane caused by the amines were recorded by an auxotonic writing lever on a kymograph. The amines and the MAO inhibitors were injected into the femoral vein.

Measurement of motility in mice. Motility was measured in mice in the motimeter described by Knoll [34]. Groups of ten mice were used. Physiological saline (controls) and the test compounds were injected in 0.2 ml volume s.c. after 2 hr habituation in the motimeter. Motility was assessed continuously after the injection for 1 hr and the total number of crossings of the whole group ("motility count") indicated the effect.

Testing the metabolic rate of rats. The method of Issekutz and Issekutz [35] was employed in rats weighing 130–180 g slightly anaesthetized with urethane (0.5 g/kg). The oxygen consumption in ml/hr was calculated as follows:

$$V \cdot \frac{P}{760} \cdot \frac{273}{273 + t} \cdot \frac{1}{T},$$

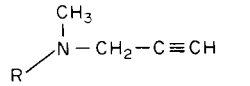
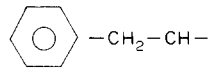
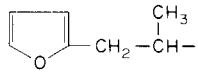
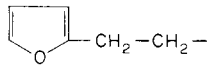
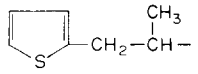
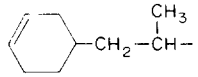
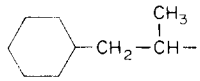
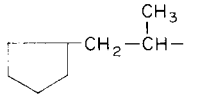
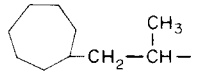
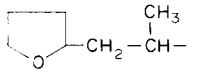
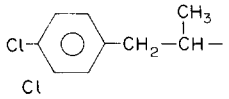
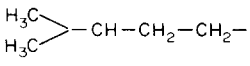
where V = volume of system (ml) = 2000 ml, P = fall in pressure within system (mmHg), t = experimental temperature, T = time of measurement (hr).

The oxygen consumption was calculated for body surface (dm^2) using the formula

$$f = 7.47 \cdot S^2,$$

where S = body wt (g). Drugs were s.c. injected thirty min after administration; their effect on oxygen consumption was estimated for a 5 min period. Oxygen consumption in the controls (235 rats) was found to be 77.6 ± 0.8 (48.9–127.2) ml/dm^2 . hr.

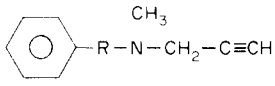
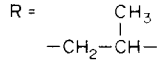
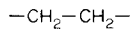
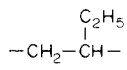
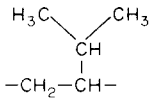
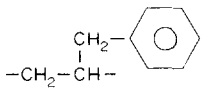
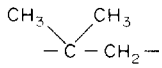
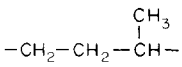
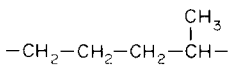
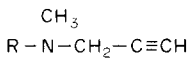
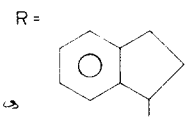
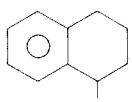
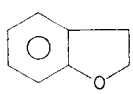
Table 1. The importance of the aromatic nature of the ring in the MAO inhibitory effect of (−)deprenyl and its analogues. Experiments with mitochondrial fraction of rat brain and rat liver

	Code no.	Preparation used	MAO inhibitory effect ID_{50} (M) Substrate:[^{14}C]tyramine Brain	Liver
R=				
	(−)deprenyl	*N*-methyl-*N*-propargyl-(2-phenyl--1-methyl)-ethyl-ammonium.HCl	5.10^{-7}	5.10^{-7}
	U-1424	*N*-methyl-*N*-propargyl-(2-furyl--1-methyl)-ethyl-ammonium.HCl	5.10^{-7}	5.10^{-7}
	TZ-945	*N*-methyl-*N*-propargyl-(2-furyl-1--ethyl-ammonium.HCl	$7{,}5.10^{-7}$	$7{,}5.10^{-7}$
	TZ-1046	*N*-methyl-*N*-propargyl-(2-thienyl--1-methyl)-ethyl-ammonium.HCl	1.10^{-4}	5.10^{-6}
	J-514	*N*-methyl-*N*-propargyl-(2-cyclo-hexyl-1-methyl)-ethyl-ammonium.HCl	$> 1.10^{-4}$	$7{,}5.10^{-4}$
	J-505	*N*-methyl-*N*-propargyl-(2-cyclo-hexyl-1-methyl)-ethyl-ammonium.HCl	$> 1.10^{-4}$	1.10^{-4}
	J-511	*N*-methyl-*N*-propargyl-(2-cyclo-pentyl-1-methyl)-ethyl-ammonium.HCl	$2{,}5.10^{-6}$	1.10^{-6}
	J-513	*N*-methyl-*N*-propargyl-(2-cyclo-heptyl-1-methyl)-ethyl-ammonium.HCl	1.10^{-4}	1.10^{-4}
	TZ-1037	*N*-methyl-*N*-propargyl-(2-tetra-hydrofuryl-1-methyl)-ethyl-ammonium.HCl	$> 1.10^{-4}$	5.10^{-5}
	J-510	*N*-methyl-*N*-propargyl-(2-/3,4--dichlorophenyl/-1-methyl)-ethyl-ammonium.HCl	1.10^{-4}	$2{,}6.10^{-6}$
	J-506	*N*-methyl-*N*-propargyl-(2-iso-butyl-1-methyl)-ethyl-ammonium.HCl	1.10^{-4}	5.10^{-5}

RESULTS

The role of the aromatic ring in the MAO inhibitory effect of (−)deprenyl and its analogues. The importance of the aromatic nature of the ring in affinity of (−)deprenyl to MAO is shown in Table 1. The furan-analogue of deprenyl (U-1424), i.e. the change of the benzene ring for another aromatic ring did not influence significantly the MAO-inhibitory effect. The introduction of a thyenyl ring (TZ-1046) reduced the MAO-inhibitory effect as compared to the efficiency of U-1424. The partial saturation of the benzene ring in deprenyl (J-514) abolished the MAO-inhibitory effect of the compound in brain and strongly reduced it in the liver. The weak effect of J-514 on liver MAO was eliminated by the complete saturation of the benzene ring (J-505). Cyclopentil (J-511) and cycloheptil (J-523) anagolues of deprenyl were also synthetized and found to be less potent than the parent compound. The same change was found with regard to U-1424. The saturation of the furan ring of U-1424 strongly decreased the MAO

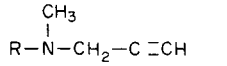
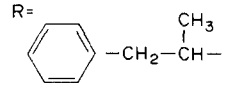
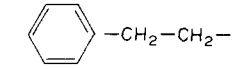
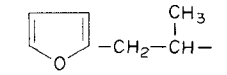
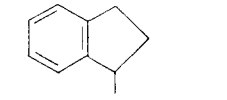
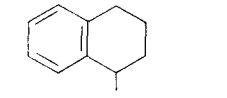
Table 2. The structural requirements of the chain between the aromatic ring and the nitrogen for the affinity of (−)deprenyl-analogues to MAO. Experiments with mitochondrial fraction of rat brain and rat liver

	Code no.	Preparation used	MAO inhibitory effect ID_{50} (M) Substrate:[^{14}C]tyramine Brain	Liver
	(−)deprenyl	*N*-methyl-*N*-propargyl-(2-phenyl-1-methyl)-ethyl-ammonium.HCl	5.10^{-7}	5.10^{-7}
	TZ-650	*N*-methyl-*N*-propargyl-(2-phenyl)-ethyl-ammonium.HCl	$7{,}5.10^{-7}$	$7{,}5.10^{-7}$
	J-501	*N*-methyl-*N*-propargyl-(2-phenyl-1-ethyl)-ethyl-ammonium.HCl	5.10^{-6}	$2{,}5.10^{-6}$
	J-502	*N*-methyl-*N*-propargyl-(2-phenyl-1-isopropyl)-ethyl-ammonium.HCl	5.10^{-5}	5.10^{-5}
	J-504	*N*-methyl-*N*-propargyl-(2-phenyl-1-benzyl)-ethyl-ammonium.HCl	$> 1.10^{-4}$	$> 1.10^{-4}$
	TZ-996	*N*-methyl-*N*-propargyl-(2-phenyl-2,2-dimethyl)-ethyl-ammonium.HCl	$> 1.10^{-4}$	$> 1.10^{-4}$
	TZ-1062	*N*-methyl-*N*-propargyl-(2-phenyl-1-methyl)-propyl-ammonium.HCl	$7{,}5.10^{-6}$	$2{,}5.10^{-6}$
	U-1520	*N*-methyl-*N*-propargyl-(2-phenyl)-pentyl-ammonium.HCl	$7{,}5.10^{-5}$	5.10^{-5}
				
	J-508	*N*-methyl-*N*-propargyl-(1-indanyl)-ammonium.HCl	1.10^{-6}	1.10^{-6}
	J-512	*N*-methyl-*N*-propargyl-(5,6,7,8-tetrahydro-1-naphthyl)-ammonium.HCl	1.10^{-6}	1.10^{-6}
	TZ-1116	*N*-methyl-*N*-propargyl-(3/2H/-benzo-furanyl)-ammonium.HCl	$> 1.10^{-4}$	$2{,}5.10^{-5}$

inhibitory effect: TZ-1037 did not inhibit brain MAO and acted as a very weak inhibitor of the liver enzyme. The important role of the aromatic ring in the affinity of (−)deprenyl to the enzyme is also shown by the low MAO-inhibitory effect of J-510 (3,4-dichlorodeprenyl) and J-506 (the compound without the benzene ring).

The role of the chain between the aromatic ring and the nitrogen in the MAO-inhibitory effect of (−)deprenyl and its analogues. Table 2 shows the structural requirements of the chain between the aromatic ring and the nitrogen for the affinity of (−)deprenyl and its analogues to MAO. It is evident from the data in this table that the ethyl-group, i.e. the unsubstituted two-carbon chain (TZ-650) is optimal for binding. Deprenyl, the C_1-methyl analogue of TZ-650 is about as potent as the parent compound. Activity decreased by the introduction of an ethyl-group at C_1 (J-501). The substitution of an isopropyl group in the same position (J-502) decreased drastically and a more bulky substitution (J-504) eliminated the MAO-inhibitory effect. Also the elongation of the chain (U-1520 and TZ-1062) decreased activity considerably. The binding to the enzyme was completely eliminated by the substitution of two methyl groups at C_2 position (TZ-966). The closing of an unsubstituted short chain to the aromatic ring, i.e. the forming of an indanyl (J-508)

Table 3. The MAO-B inhibitory effect of new (−)deprenyl analogues. Experiments with mitochondrial fraction of rat brain and liver

	Code no.	MAO inhibitory effect ID_{50} (M) Substrate:[^{14}C]PEA Brain	Liver
	(−)deprenyl	$7{,}5.10^{-7}$	5.10^{-7}
	TZ-650	1.10^{-5}	5.10^{-6}
	U-1424	1.10^{-5}	5.10^{-6}
	J-508	1.10^{-7}	$2{,}5.10^{-7}$
	J-512	$2{,}5.10^{-7}$	$2{,}5.10^{-6}$

or tetrahydronaphthyl ring (J-512) yields potent MAO-inhibitors, while the formation of a benzofuranyl ring (TZ-1116) strongly reduced activity.

The selectivity of the new (−)deprenyl-derived inhibitors towards MAO-B (phenylethylamine oxidase). (−)Deprenyl is a very effective inhibitor of the oxidation of PEA, the only endogenous amine, specific to MAO-B. Table 3 shows that using mitochondrial preparations and [^{14}C]PEA as substrate, TZ-650 and U-1424 were found to be less potent, J-508 more potent than (−)deprenyl. Also J-512 inhibited brain MAO-B more potently than (−)deprenyl but was much less effective against the liver enzyme.

In a previous study a new method for checking selective MAO-B inhibitors *in vivo* was described in the cat [24]. The essence of this method lies in the fact that PEA and 5-HT are metabolized in the liver and changes in the blood concentrations of these amines can be checked continuously and with high sensitivity by observing the nictitating membrane as this organ contracts dose-dependently under the influence of PEA and 5-HT, respectively. As 5-HT is metabolized by MAO-A and PEA by MAO-B, the selectivity *in vivo* of an MAO inhibitor can be easily checked by this method. Selective inhibitors of MAO-B, like (−)deprenyl, inhibit the metabolism of PEA in doses which do not influence the catabolism of 5-HT. Figures 1 and 2 demonstrate that both J-508 and U-1424 are potent selective inhibitors of MAO-B. The i.v. dose of 0.25 mg/kg of these compounds shifted the dose response curve of PEA to the left leaving the effect of 5-HT unchanged.

In a series of experiments we analyzed in detail the effects *in vivo* of (−)deprenyl, U-1424 and J-508 on the MAO-A and B in liver and brain. The inhibitors were i.v. injected in different doses 1 hr prior to the removal of the brain and liver for the assay of enzyme activity. As substrates 5-HT (specific for MAO-A), PEA (specific for MAO-B) and tyramine (common substrate for both enzymes) were used. The results are presented in Tables 4–6. In the rat J-508 proved to be the most potent inhibitor *in vivo*. Regarding the selectivity towards MAO-B, U-1424 seems to be the most favourable.

Deprenyl, a derivative of methamphetamine is not completely devoid of the characteristic central nervous system (CNS) effect of the parent compound. This was analyzed in detail previously [2, 3]. We also showed that as in the case of methamphetamine the stimulatory effects of (+)deprenyl on the CNS is more pronounced than that of the (−)form [5]. The change of the aromatic ring for a furan ring caused a further decrease in CNS stimulating effect. This is shown in Tables 7 and 8. As it can be seen, a very high dose of (−)deprenyl (20 mg/kg, s.c.) significantly enhanced the spontaneous motility of mice (Table 7) and also considerably increased the oxygen consumption of rats (Table 8), while the same amount of U-1424 was slightly depressant in the motility test, and the high dose of U-1424 (20 mg/kg) was less potent than (−)deprenyl in increasing oxygen consumption in the rat. For the sake of comparison, the effects of amphetamine, racemic and (+)deprenyl are also shown in Tables 7 and 8. It is evident that even (+)deprenyl which is more potent than (−)deprenyl in both tests can be considered as a weak stimulant compared to methylamphetamine and amphetamine, respectively.

Table 9 shows that the toxicity of U-1424 in the rat is similar to that of (−)deprenyl.

DISCUSSION

Since the first demonstration of the efficacy of iproniazid in psychotic depression and the observation that when this drug is used in psychiatry pos-

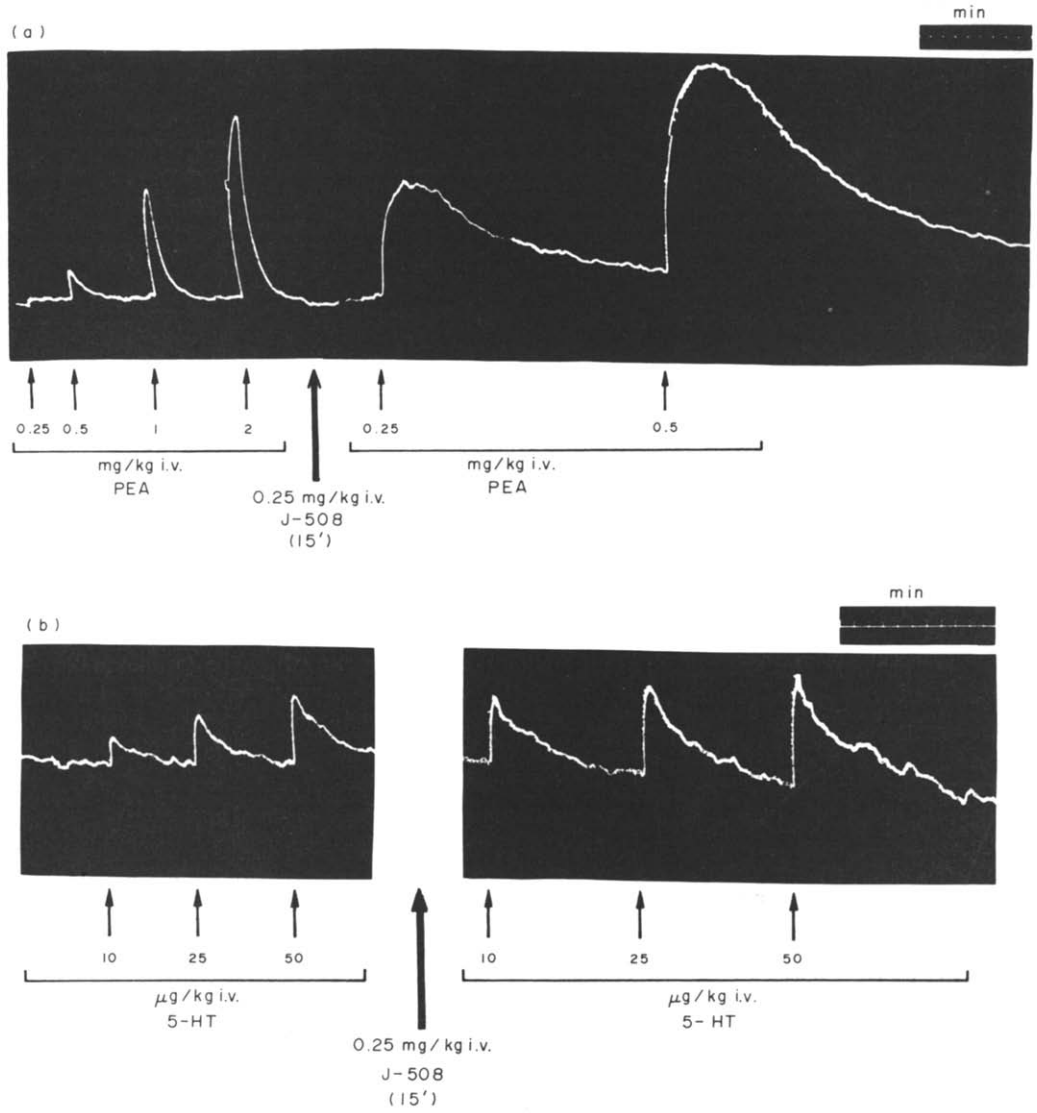


Fig. 1. The dose-dependency of the phenylethylamine (PEA)-induced contractions of the nictitating membrane and the increase of the effects of PEA 15 min after i.v. administration of 0.25 mg/kg J-508. The dose-dependency of the serotonin (5-HT)-induced contractions of the nictitating membrane and the potentiation of the effects of 5-HT 15 min after i.v. administration of 0.25 mg/kg J-508. Weight of the cat, 3 kg. Chloralose urethane anaesthesia. Artificial respiration.

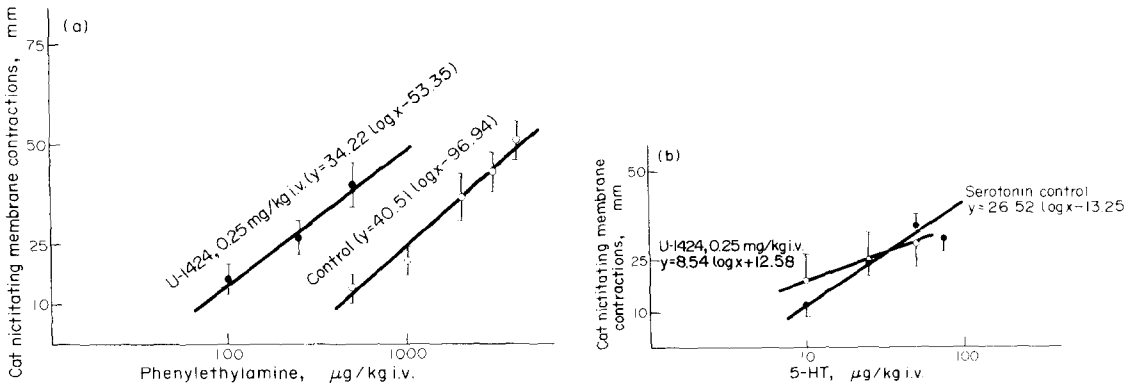


Fig. 2. Phenylethylamine (PEA)-induced dose-related nictitating membrane contractions and the potentiation of the effects by U-1424 (0.25 mg/kg, i.v.). The ineffectiveness of U-1424 (0.25 mg/kg, i.v.) on the serotonin (5-HT) induced dose-related nictitating membrane contractions. Each point indicates the average of experiments on four cats.

Table 4. The MAO-inhibitory effect of intravenously injected (−)deprenyl, J-508 and U-1424 in the rat using [^{14}C]-tyramine as substrate

Dose mg/kg	(−)Deprenyl		J-508		U-1424	
	Brain	Liver	Brain	Liver	Brain	Liver
0.01	9.4 ± 1.3	3.4 ± 1.2	—	—	—	—
0.1	14.5 ± 2.3	17.6 ± 3.5	25.3 ± 4.1	44.4 ± 4.4	6.3 ± 1.9	17.3 ± 5.61
1.0	59.0 ± 6.3	70.5 ± 2.6	71.6 ± 7.2	79.3 ± 7.2	18.3 ± 1.9	23.6 ± 2.5
5.0	65.1 ± 5.4	72.9 ± 1.8	86.1 ± 4.1	97.3 ± 0.5	47.2 ± 2.0	42.4 ± 2.8
10.0	80.6 ± 7.6	76.0 ± 0.5	85.6 ± 1.3	98.7 ± 0.2	59.4 ± 1.9	56.7 ± 4.7
25.0	95.6 ± 1.6	94.7 ± 7.3	91.2 ± 2.1	98.2 ± 1.0	70.4 ± 1.8	77.2 ± 1.8

Inhibition of MAO in brain and liver homogenates is expressed in percentage of the control ± S.E.M.
Rats were decapitated 1 hr after drug administration.

Table 5. The MAO-inhibitory effect of intravenously injected (−)deprenyl, J-508, and U-1424 using [^{14}C]PEA as substrate

Dose mg/kg	(−)Deprenyl		J-508		U-1424	
	Brain	Liver	Brain	Liver	Brain	Liver
0.01	15.4 ± 8.5	8.7 ± 2.1	—	—	—	—
0.1	55.9 ± 7.5	35.7 ± 6.5	73.7 ± 5.4	41.7 ± 3.1	45.4 ± 4.9	29.4 ± 2.5
1.0	76.6 ± 6.4	66.0 ± 5.8	91.9 ± 4.6	79.4 ± 1.3	74.7 ± 2.4	57.7 ± 1.3
5.0	87.5 ± 5.2	79.8 ± 2.9	92.2 ± 3.2	91.0 ± 1.1	78.7 ± 4.2	77.8 ± 1.5
10.0	94.4 ± 2.2	82.4 ± 1.1	99.8 ± 0.2	95.0 ± 1.1	82.2 ± 4.8	85.4 ± 4.5

Inhibition of MAO in brain and liver homogenates is expressed in percentage of the control ± S.E.M.
Rats were decapitated 1 hr after drug administration.

Table 6. The MAO inhibitory effect of intravenously injected (−)deprenyl, J-508 and U-1424 using [^{14}C]5-HT as substrate

Dose mg/kg	(−)Deprenyl		J-508		U-1424	
	Brain	Liver	Brain	Liver	Brain	Liver
0.01	4.1 ± 2.1	8.3 ± 4.3	—	—	—	—
0.1	9.5 ± 2.7	11.8 ± 4.5	33.1 ± 2.9	11.1 ± 0.7	7.2 ± 0.8	3.1 ± 1.1
1.0	22.6 ± 5.4	14.9 ± 4.4	89.5 ± 1.2	40.1 ± 8.3	36.3 ± 2.4	7.8 ± 2.9
5.0	53.8 ± 4.8	31.1 ± 6.8	95.5 ± 0.6	78.4 ± 0.7	43.1 ± 0.5	24.3 ± 4.5
10.0	71.8 ± 2.2	36.6 ± 8.3	95.4 ± 1.6	95.4 ± 1.2	59.7 ± 1.7	40.1 ± 2.8

Inhibition of MAO in brain and liver homogenates is expressed in percentage of the control ± S.E.M.
Rats were decapitated 1 hr after drug administration.

Table 7. Comparison of the effects of deprenyl and U-1424 on the spontaneous motility of mice

	Dose mg/kg	Motility counts* in the motimeter
Physiological saline	—	311
(±)Methamphetamine	1	3192
	2	6085
(+)Deprenyl	2	695
	10	1958
	20	2212
(−)Deprenyl	2	508
	10	702
	20	1185
U-1424	20	105

* Number of crossings of a group of ten mice in the motimeter within 1 hr after the s.c. injection of the drug. Habituation period before drug treatment; 2 hr.

Table 8. Comparison of the effects of deprenyl and U-1424 on the metabolic rates of rats

Drug	Dose mg/kg	Change in the basal metabolic rate in rats* Groups of twelve rats (%)
Amphetamine	2	+51
	10	+105
(±)Deprenyl	5	+17
	20	+47
	30	+114
(+)Deprenyl	20	+66
(−)Deprenyl	20	+43
U-1424	5	−10
	20	+28

* Basal metabolic rate in control rats ($n = 235$) = 77.6 ± 0.8 (48.9–127.2) ml/dm^2.hr.

Table 9. Comparison of the toxicity of (−)deprenyl and U-1424

Drug	Route of administration	LD_{50} mg/kg
(−)Deprenyl	i.v.	82 (71.9–92.1)
	s.c.	240 (206.8–273.2)
	oral	345 (215.6–474.4)
U-1424	i.v.	104 (88.6–119.4)
	s.c.	168 (120.0–216.0)

Values in brackets indicate 95 per cent confidence limits

tural hypotension is a frequent side effect, the therapeutic use of MAO inhibitors have been related to their effects in the CNS and cardiovascular system. The occasionally fatal acute hypertensive reaction following the administration of MAO inhibitors because of potentiation with a variety of pressor drugs, indirectly acting sympathomimetics and food substances containing vasoactive compounds, undermined confidence in the MAO inhibitors.

The potentiation of the pressor effect of tyramine seems to play the main role in the dangerous hypertensive reactions following the intake of certain food materials containing high amounts of free tyramine (e.g. different cheeses, yeast products, beans, Chianti wines, pickled herring, chicken liver, etc.) The "cheese reaction", first described by Blackwell in 1963 [36], is now known to be primarily a consequence of the inhibition of intestinal MAO. The direct noradrenaline-releasing effect of tyramine is also enhanced. (−)Deprenyl is safe with respect to the hazards involved in combination with a variety of food and drugs, because in contrast to the non-selective and A-selective MAO inhibitors.

(1) It is a poor inhibitor of the MAO activity of the intestine [9, 37], which by splitting the pressor amines (e.g. tyramine) of foodstuffs controls their access to the circulation.

(2) It does not enhance, rather inhibits the release of [^{3}H] noradrenaline [5].

(3) It does not potentiate, rather inhibits the pressor effect of tyramine [4].

In good agreement with our findings in animals, Varga and Tringer [38] and Tringer *et al.* [39] never noticed the appearance of hypertensive reaction during their clinical trials, using racemic or (−)deprenyl as antidepressants. Neither Birkmayer *et al.* [40] who administered (−)deprenyl for 2 yr, nor Lees *et al.* [41] mentioned this type of side effect.

Our claim [24] that the administration of (−)deprenyl is free from the "cheese effect" has received substantial experimental backing in a recent study in man [42].

As the dopaminergic nerve terminals in the striatum in man probably contain MAO-B [43], the peculiar spectrum of activity of selective MAO-B inhibitors is of practical importance for the levodopa treatment of Parkinson's disease. At present the use of MAO inhibitors is considered to be definitely contraindicated in patients treated with levodopa, because hypertension may result [44]. As the selective MAO-B inhibitors are devoid of this type of side effect, the application of this potentially valuable levodopa-sparing mechanism became feasible. Recent clinical studies [40, 41] have clearly demonstrated distinct advantages of therapy with the use of (−)deprenyl concurrently with levodopa or Madopar in Parkinsonian patients.

The structure–activity relationship study presented in this paper seems to be not only of theoretical interest in the light of the promising new therapeutic aspects of the selective MAO-B inhibitors. (−)Deprenyl preserved a very low CNS stimulatory effect reminiscent of that of methamphetamine, the parent molecule. Even this slight effect was eliminated by changing the nature of the aromatic ring. Further studies with the newly developed selective MAO-B inhibitors are now planned to find out their therapeutic value.

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