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DOES THE B FORM SELECTIVE MONOAMINE OXIDASE INHIBITOR LOSE SELECTIVITY BY LONG TERM TREATMENT?

BERTIL EKSTEDT*, KÁLMÁN MAGYAR and JOSEPH KNOLL
Department of Pharmacology, Semmelweis Medical University, Budapest, Hungary

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Abstract—Rats were treated subcutaneously with different doses of the "B form" selective monoamine oxidase (MAO) inhibitors deprenyl (phenylisopropylmethylpropargylamine), U-1424 (*N*-methyl-*N*-propargyl-[2-furyl-1-methyl]-ethylammonium) and J-508 (*N*-methyl-*N*-propargyl-[1-indenyl]-ammonium. HCl) in order to study the changes of their selectivity during 21 days of treatment. When the daily dose of (–) deprenyl and U-1424 was 0.05 or 0.25 mg/kg body wt (similar to the human dosage of deprenyl in clinical trials), in spite of their repeated administration, a fairly selective inhibition pattern was maintained. In this case at a low rate of oxidation of beta-phenylethylamine (MAO-B form specific substrate) the conversion of serotonin (MAO-A form specific substrate) was near to the untreated value. When 1.0 mg/kg of these inhibitors were repeatedly administered they also inhibited the A form of MAO.

As J-508 is a more potent MAO inhibitor than deprenyl and U-1424, even the lowest dose (0.05 mg/kg) used in this study proved to inhibit MAO-A. All the compounds tested were less effective on liver than on brain MAO; thus their selectivity was more pronounced on liver homogenate.

Hypertensive crisis is the most serious toxic effect of monoamine oxidase (MAO) inhibitors related either to drug interaction or to the ingestion of foods containing enough tyramine ("cheese effect"). Deprenyl, a new spectrum MAO inhibitor developed by Knoll *et al.* [1, 2] was shown to be a selctive B-type blocker [3], and a weak inhibitor of the mainly A-type MAO in the intestines [4, 5]. It is a potent antagonist to the effect of tyramine [6] and is considered to be safe with respect to the hazards of combination with a variety of foods and drugs [7]. This approach was substantially supported in a recent study showing that huge amounts of tyramine failed to raise blood pressure in men pretreated with (—)deprenyl [8].

The demonstration that selective MAO-B inhibitors are safer than the non-selective ones hitherto used [7] is of great practical importance. Clorgyline, the selective inhibitor of the A-type MAO as well as the non-selective inhibitors used in therapy are definitely contraindicated in Parkinsonian patients on levodopa because hypertension may result [9]. (—)Deprenyl, however, was found to be a useful drug in Parkinsonism as it could be administered concurrently with levodopa without side effects [10, 11].

In planning experiments with selective MAO-B inhibitors, it may be overlooked that these compounds lose selectivity in higher doses. This paper is devoted to demonstrating that the maintenance of the selective inhibition of the B form of MAO during a long term treatment with selective inhibitors needs carefully selected doses. (—)Deprenyl and two of its newly developed structural analogues *N*-methyl-*N*-propargyl-[1-indenyl]-ammonium.HCl (J-508) and *N*-methyl-*N*-propargyl-[2-furyl-1-methyl]-ethylammonium (U-1424), which were found to be potent selective inhibitors of MAO-B both *in vitro* and *in vivo* [12, 13], were used in this study.

MATERIALS AND METHODS

Materials (—)Deprenyl (phenylisopropylmethylpropargylamine hydrochloride), U-1424 (*N*-methyl-*N*-propargyl-[2-furyl-1-methyl]-ethylammonium) and J-508 (*N*-methyl-*N*-propargyl-[1-indenyl]-ammonium. HCl) were synthesised by Chinoin, Budapest.

Treatment of rats. Male Wistar CFY rats weighing at least 110 g at the beginning of the experiments were injected subcutaneously with 0.2 ml of different concentrations of (—)deprenyl, U-1424 or J-508 daily. For comparison single dose experiments were also carried out. The rats were decapitated 4 hr after the last injection. For each of the treated groups, a control one of the same age was decapitated on the same day. At the end of the treatment the weights of the rats were between 150 and 300 g (average 215 g).

Preparation of homogenates. After decapitation, the rats were bled and the livers and brains removed, weighed and chilled. The tissues were homogenized in 10 vol. of 0.25 M sucrose in a Potter–Elvehjem homogeniser. The final protein concentration in the liver homogenate was 17.9 mg/ml and in the brain homogenates 11.5 mg/ml.

Assay of MAO activity and expression of the results. The radiometric assay of Wurtman and Axelrod [14] as modified by Magyar and Knoll [15] was essentially used. However, the final concentration of [^{14}C]beta-phenylethylamine ([^{14}C]PEA) was 50μM and of [^{14}C]serotonin ([^{14}C]5-HT) 500μM. All the results are expressed as per cent of the MAO activity in the corresponding control group of rats. The mean values of the MAO A form activities (serotonin as a substrate) in the different control groups ranged from 0.44 to

* Permanent address: University of Umea, Department of Pharmacology, S-901 87, Umea, Sweden.

Table 1. The activity of the A and B forms of monoamine oxidase (MAO) in rat brain and liver homogenates after daily subcutaneous treatment with (—)deprenyl

Mode of administration	Brain						Liver					
	PEA 0.05 mg/kg	5-HT 0.05 mg/kg	PEA 0.25 mg/kg	5-HT 0.25 mg/kg	PEA 1.0 mg/kg	5-HT 1.0 mg/kg	PEA 0.05 mg/kg	5-HT 0.05 mg/kg	PEA 0.25 mg/kg	5-HT 0.25 mg/kg	PEA 1.0 mg/kg	5-HT 1.0 mg/kg
Daily doses												
1 day	52.5	97.5	35.3	95.8	27.6	85.0	77.4	83.9	62.5	83.4	35.6	89.2
7 days	24.3	93.8	19.9	87.2	14.3	56.2	62.4	84.6	37.6	108.2	22.2	100.7
14 days	24.3	92.7	18.3	75.6	9.3	35.6	61.9	94.3	48.4	109.0	17.9	99.8
21 days	23.2	81.2	16.6	75.0	8.7	31.5	69.1	98.7	42.2	101.2	19.1	85.2
Accumulated dose (21 × single dose) single inj.	—	—	14.9*	46.2*	1.5+	7.4+	—	—	10.6*	63.5*	5.7+	52.8+

The values are expressed as per cent of the MAO activity for untreated rats of the same age. Each value is the mean for 3 rats.
* Dose: 5.25 mg/kg body weight.
+ Dose: 21.0 mg/kg body weight.
The accumulated dose after daily injections of 0.05 mg/kg for 21 days (1.05 mg/kg) corresponds well to the single injection of 1.0 mg/kg.

Table 2. The activity of the A and B forms of monoamine oxidase (MAO) in rat brain and liver homogenates after subcutaneous treatment with (—)deprenyl

Total dose: 5.25 mg/kg	Brain		Liver	
Single dose: 1.75 mg/kg injected once a week, three times	PEA	5-HT	PEA	5-HT
Killed 4 hr after last injection	14.5	61.1	25.3	91.6
Killed 7 days after last injection	49.9	71.6	79.5	94.6

The values represent the per cent of the MAO activity for untreated rats of the same age. Each value is the mean for 3 rats.

0.55 μmol/g wet wt/min and from 1.13 to 1.32 μmol/g wet wt/min in the brain and in the liver, respectively. For the B form (beta-phenylethylamine as a substrate), they varied between 0.11 and 0.15 μmol/g wet wt/min in the brain and between 0.78 and 0.98 μmol/g wet wt/min in the liver.

Protein. Protein was estimated according to Lowry *et al.* [16] with human serum albumin as a standard.

RESULTS

The effect of treatment of rats with (—)deprenyl is shown in Table 1. After 7 days of treatment with a daily dose of 0.05 mg/kg body weight, only a minor effect on the A form of the enzyme in brain, as shown by the activity towards serotonin, could be detected, but the activity of the B form (the activity towards beta-phenylethylamine) was reduced to about 25 per cent. At the highest dose (1.0 mg/kg) of (—)deprenyl, however, the selectivity was less pronounced in brain. In the liver the activity of both the A and B forms was at all doses less inhibited than in the brain. Even when 1.0 mg/kg was given, almost all of the A form activity in the liver remained, while only about 20 per cent of the B form activity could be recorded, showing a high degree of selective inhibition even with this dose. In the brain at the same dose, about 30 per cent of the A form activity and less than 10 per cent of the B form activity remained, which means that the selectivity in this tissue was less than in the liver at 1.0 mg/kg. After 14 or 21 days of treatment the activities were not significantly more inhibited than after 7 days at any dose, neither in the liver nor in the brain, with the exception of the A form activity in the brain at the highest dose. If only one single dose was given, the degree of inhibition was less than after repeated doses. On the other hand, when the total dose given for 21 days was injected on one single occasion, a considerably higher degree of inhibition occurred than when given daily, and a great deal of the selectivity was lost.

To study how weekly doses of (—)deprenyl influence the MAO activity, rats were given 1.75 mg/kg of (—)deprenyl on three different occasions 7 days apart. The total dose corresponded to 0.25 mg/kg given daily for 21 days. One of the groups was killed 4 hr after the last injection and another group was kept 7 days after that injection. In Table 2 it can be seen that the B form activity was considerably less, immediately after the last injection of the weekly dose, than after the same total dose given daily (Table 1) both in the brain and in the liver. After 7 days without treatment a great deal of both the A and the B form activity in both tissues was recovered (Table 2).

U-1424 seems to have about the same potency as (—)deprenyl in the liver but a slightly less pronounced effect in the brain (Table 3). As for (—)deprenyl, the selectivity was well retained in both tissues even after 21 days of treatment. When the total dose for 21 days was given in one single injection, the degree of inhibition was higher and the selectivity less than when given in daily doses.

J-508 was found to have a higher potency than (—)deprenyl and U-1424 both in the liver and in the brain (Table 4). At a daily dose of 0.25 mg/kg most of both the A and B form activity was inhibited in the brain. As can be seen for instance in the brain, when a dose of 0.05 mg/kg was used, the selectivity for the B form was much less than with the other compounds. This was also the case in single dose experiments.

DISCUSSION

(—)Deprenyl has a higher affinity to the B form of MAO than to the A form [3]. At high concentration, or when the enzyme is exposed to the inhibitor for a long time, the A form is also inhibited. As a MAO inhibitor of the acetylenic type, (—)deprenyl interacts covalently with the active site of the enzyme, performing irreversible inhibition [17]. The irreversible inactivation of the enzyme is, however, preceded by a reversible phase [15]. On account of this the question must be considered whether (—)deprenyl loses its selectivity to the B form when patients are treated daily with the drug. The experiments described here, however, clearly show that the selectivity of (—)deprenyl is about the same when rats are treated with repeated doses for 21 days as in single dose experiments.

The uptake of deprenyl in brain is a fast procedure reaching the peak concentration within seconds after an intravenous dose and almost all of the inhibitor is eliminated from this tissue after 20–30 min [18]. Thus, it seems likely that the enzyme is not exposed to the inhibitor in brain for a long enough time and at a high enough concentration to establish irreversible bindings to the A form after each injection of the lowest dose, used here. At the highest dose, (—)deprenyl is reaching a concentration in the brain high enough to inhibit a portion of the A form too. The inhibition of both the A and B forms is higher in the brain than in the liver which may also be explained by the fast penetration of (—) deprenyl into the brain, rendering a higher concentration in this tissue in the initial phase of its distribution.

If (—)deprenyl was given in one single dose instead of in daily doses, but with the same total amount of the inhibitor, a higher degree of inhibition occurred, which means that a large proportion of both the A and B forms has been resynthesised during the period of treatment. No difference in the degree of inhibition at the two lowest doses occurred when rats were treated for 14 or 21 days compared to the treatment for 7 days, which means that the daily synthesis of the enzyme was as high as the inhibition after each dose. The major portion of the inhibited activity was restored one week after treatment with three weekly injections of (—)deprenyl (Table 2), which shows a great fluctuation of the enzyme activity when weekly doses are given instead of daily doses.

We developed novel deprenyl-derived selective inhibitors of MAO recently [12, 13, 15]. Of the new structural analogues of deprenyl, U-1424 and J-508 were selected for this study. In U-1424 the benzene ring of deprenyl has been changed for a furan ring and J-508 is the indenyl analogue of deprenyl. U-1424 was found to have about as high selectivity as deprenyl to the B form of MAO (Table 3) but the selectivity of J-508 is somewhat less pronounced. The latter inhibitor has, however, a higher potency than both U-1424 and (—) deprenyl, and accordingly the selectivity is lost at a lower dose than for the other inhibitors. The compounds do not lose their selectivity during treatment for 14 or 21 days, but after a single injection of a high

Table 3. The activity of the A and B forms of monoamine oxidase (MAO) in rat brain and liver homogenates after daily subcutaneous treatment with U-1424

Mode of administration	Brain						Liver					
	PEA 0.05 mg/kg	5-HT 0.05 mg/kg	PEA 0.25 mg/kg	5-HT 0.25 mg/kg	PEA 1.0 mg/kg	5 HT 1.0 mg/kg	PEA 0.05 mg/kg	5-HT 0.05 mg/kg	PEA 0.25 mg/kg	5-HT 0.25 mg/kg	PEA 1.0 mg/kg	5-HT 1.0 mg/kg
Daily doses												
1 day	59.6	97.5	46.4	101.1	27.4	90.8	80.0	104.9	74.8	86.3	41.0	98.5
7 days	41.6	100.1	29.1	89.3	20.5	68.6	75.3	94.7	43.0	87.0	23.4	93.6
14 days	41.5	96.3	25.3	79.5	13.0	48.8	70.6	105.1	38.6	93.6	19.6	83.4
21 days	40.4	98.4	22.5	81.9	13.6	46.5	67.5	90.2	45.9	106.3	19.9	81.4
Accumulated dose (21 × single dose), single inj.	—	—	16.1*	51.5*	5.2+	19.5+	—	—	11.7*	68.9*	6.3+	44.1*

*+ For details see Table 1.

Table 4. The activity of the A and B forms of monoamine oxidase in rat brain and liver homogenates after daily subcutaneous treatment with J-508

Mode of administration	Brain						Liver					
	PEA 0.05 mg/kg	5-HT 0.05 mg/kg	PEA 0.25 mg/kg	5-HT 0.25 mg/kg	PEA 1.0 mg/kg	5-HT 1.0 mg/kg	PEA 0.05 mg/kg	5-HT 0.05 mg/kg	PEA 0.25 mg/kg	5-HT 0.25 mg/kg	PEA 1.0 mg/kg	5-HT 1.0 mg/kg
Daily doses												
1 day	45.4	94.0	18.3	62.2	2.0	4.5	89.7	104.9	51.4	99.1	10.6	43.4
7 days	29.1	54.3	4.4	9.8	6.5	1.3	41.0	91.9	28.0	72.1	7.0	25.1
14 days	12.8	39.9	2.5	8.0	0.5	0.4	61.3	107.8	19.3	70.6	5.1	22.1
21 days	8.3	28.7	1.3	6.3	0.1	1.8	47.1	85.6	12.2	51.0	3.6	15.6
Accumulated dose (21 × single dose), single inj.	—	—	4.8*	3.3*	0.7+	1.0+	—	—	2.8*	8.7*	0.5+	3.1*

*+ For details see Table 1.

concentration of the inhibitors a large proportion of the A form activity was also inhibited.

When patients with Parkinson's disease have been treated with (—)deprenyl, daily doses of 5–10 mg have usually been used [10, 11]. The doses correspond to about the lowest dose (0.05 mg/kg) in the present experiments, and thus, at least in the rat, the selectivity of the inhibition is good enough after treatment for 21 days (Table 1). The A form of the enzyme in the brain is slightly inhibited at this dose (81.8 per cent of control activity remained); however, the activity of the B form is only about 20 per cent of the control. When about five times the dose was administered (0.25 mg/kg body weight), the inhibition pattern of MAO concerning selectivity was the same after 3 weeks of treatment (75 per cent A and 16.6 per cent B activity remained) showing that (—)deprenyl has a satisfactory therapeutic range.

Regarding the inhibitory properties of U-1424, no change in its selectivity, and only a slight difference in its potency, were recognized compared to (—)deprenyl. It has to be considered, however, that the racemic form of U-1424 was used in this study, and that this is probably less potent than the (—)form as an inhibitor of MAO activity.

J-508 was found to be a MAO inhibitor of unusually high potency. To maintain a selective inhibition of MAO-B with this compound during long term treatment, one tenth of the dose of (—)deprenyl or less has to be administered. The extremely high potency of this inhibitor of MAO might give it advantages as a drug.

The results clearly demonstrate that with carefully selected doses the selective inhibition of the B form of MAO can be found maintained during a long term treatment with (—)deprenyl and related substances. As they are irreversible inhibitors of MAO, a permanent regenerating process renewing the MAO enzyme may play an essential role in this mechanism.

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