evoked release of noradrenaline, and suggest that this effect may be due to a diminished content of the neurotransmitter in the nerve endings. Since the blood levels of lithium in patients chronically treated with lithium chloride have been reported to be about 1 mM (Schou 1976), it is suggested that only the slight effect observed with the lower concentration of the ion tested in the present study may be of clinical significance in terms of the mechanism of action of lithium.

This work was supported by a grant from the National Research Council.

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J. Pharm. Pharmacol. 1989, 41: 205–208
Communicated August 3, 1988

Deamination of aliphatic amines of different chain lengths by rat liver monoamine oxidase A and B

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Abstract—Monoamines with from 1 to 18 straight chain carbon atoms have been analysed as rat liver monoamine oxidase substrates. Methylamine and ethylamine are clearly not substrates of monoamine oxidase (MAO). n-Propylamine, n-butylamine, n-dodecylamine and n-octadecylamine are relatively poor substrates, i.e. with high K_m and low V_{max} values for the enzyme. n-Pentylamine, n-hexylamine, n-heptylamine, n-octylamine, n-nonylamine and n-decylamine are all very good MAO substrates. All these aliphatic amines are found to be typical type B substrates according to the sensitivities of the enzyme towards the selective MAO-B inhibitor selegiline and the MAO-A inhibitor, clorgyline. The sensitivity towards selegiline with respect to these amines is even higher, i.e $K_i = 1 \times 10^{-9}$ M for butylamine, than that of the typical type B substrate β-phenylethylamine ($K_i = 1 \times 10^{-8}$ M). The sensitivity towards selegiline decreases slightly with increasing chain length of these aliphatic amines.

Monoamine oxidase (MAO, EC 1.4.3.4.) is well known for its catalytic activities on endogenous aromatic monoamine substrates, such as neuronal catecholamines, indoleamines and trace amines (Blaschko 1974). The enzyme is also responsible for the detoxification of xenobiotic amines. Two types of enzymes, namely MAO-A and MAO-B, are classified according to substrate preference (i.e. type A substrate 5-hydroxytryptamine and type B substrate β-phenylethylamine) and to sensitivity towards selective MAO inhibitors, such as clorgyline (MAO-A inhibitor) and selegiline (L-deprenyl) (MAO-B inhibitor) (Fowler et al 1978; Denney & Denney 1985). It has been shown earlier in man that after administration of short chain aliphatic amines methylamine was totally metabolized and excreted as urea, whilst about one-third of ethylamine and less than 10% of propylamine and n-butylamine were recovered unchanged in the urine (Rechenberger 1940). It was later found by Blaschko (1952) that short chain aliphatic amines can be oxidized by rabbit liver MAO, and by von Korff & Wolfe (1984) that monoamines of 8 to 12 carbon atoms were also substrates for beef liver MAO. These latter amines can act as time-dependent reversible MAO inhibitors. n-Pentylamine has been found to be deaminated by MAO-A and B, as well as by semicarbazide sensitive amine oxidase in the rat heart (Guffroy et al 1983).

Recently, aliphatic amine derivatives, such as 2-n-pentylaminoacetamide, have been found to possess anticonvulsant activity (de Varebeke et al 1987). This compound crosses the blood brain barrier and is then oxidized by MAO to form glycinamide which is subsequently cleaved to glycine, which presumably exerts pharmacological activity (de Varebeke et al 1987). It is therefore necessary to understand the nature of the oxidation of aliphatic amines by MAO. In the present paper the kinetics of the deamination of unbranched aliphatic amines containing from 1 to 18 carbon atoms by both MAO-A and MAO-B has been analysed.

Materials and methods

Materials. Methylamine HCl, ethylamine HCl, n-propylamine HCl, n-butylamine, n-amylamine, n-hexylamine, n-heptylamine, n-octylamine, n-nonylamine, n-decylamine, n-dodecylamine, putrescine, cadaverine 2HCl, amagtine, homovanillic acid and horseradish peroxidase were purchased from Sigma (St. Louis, MO); octadecylamine was obtained from Aldrich (Milwaukee, WI) while selegiline (L-deprenyl) and clorgyline were gifts provided by Professor Knoll, Budapest, and May and Baker Ltd. (Dagenham, UK), respectively. All other chemicals were of analytical grade.

Preparation of rat liver monoamine oxidase. Freshly dissected rat liver tissues were rinsed with chilled 0·9% NaCl (saline), cut into small pieces, and homogenized immediately in ice-cold 0·32 M sucrose in 0·01 M phosphate buffer (pH 7·5). Mitochondrial fractions were obtained by differential centrifugation (Yu 1986). Mitochondrial membrane fragments were prepared by lysing the mitochondria in chilled distilled water followed by centrifugation at 105 000 *g* for 30 min. The membrane preparations were then washed by resuspension in distilled water and recentrifuged. MAO-A and MAO-B were obtained by incubation of the MAO preparations with selegiline (1×10^{-6} M) and clorgyline (5×10^{-7} M) for 60 min at ambient temperatures, respectively. The MAO inhibitors were then separated by gel filtration on Pharmacia PD-10 disposable columns.

Assay of MAO activity. A sensitive fluorometric method based on the formation of an intense fluorescence from homovanillic acid and the hydrogen peroxide released during the oxidation of the amines was used (Snyder & Hendley 1968; Yu & Boulton 1980). The mitochondrial MAO preparations (40–50 μg protein) were incubated at 37°C for 10 min in the presence of amine substrate in a total volume of 200 μL of 0·05 M phosphate buffer (pH 7·5) containing 50 μg homovanillic acid and 0·82 units of horseradish peroxidase. The fluorescence intensity was measured in a spectrophotofluorometer (Aminco-Bowman) at an excitation wavelength of 315 nm and emission wavelength of 425 nm. The enzyme preparation does not contain detectable catalase activity which may interfere with the assay. Addition of a catalase inhibitor, sodium azide did not improve the assay condition.

Protein concentration was determined by Lowry's method (Lowry et al 1952).

The kinetic parameters were analysed according to Wilkinson (Wilkinson 1961).

Results and discussion

Although it has been reported that aliphatic amines can be deaminated by MAO (Blaschko 1952; von Korff & Wolfe 1984), it is not clear how well these amines act as MAO substrates compared with the well known aromatic amine substrates;

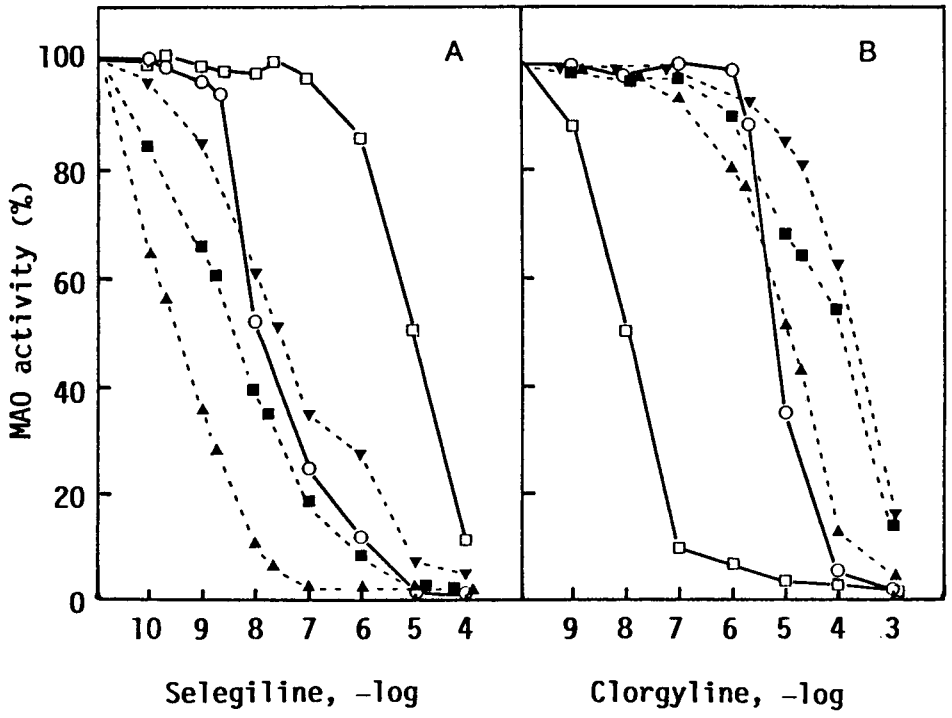

FIG. 1. Inhibition of the deamination of n-butylamine (▲----▲), n-hexylamine (■----■), n-decylamine (▼----▼), β-phenylethylamine (○——○) and 5-hydroxytryptamine (□——□) by: Panel A, clorgyline and Panel B, selegiline. Rat liver mitochondrial membrane fragments (30–45 μg) were incubated for 30 min at room temperature with the appropriate inibitor concentration before being assayed for activity. Values are given as mean of duplicate experiments of the percentage of MAO activity remaining with respect to samples preincubated with distilled water. The enzyme activities were assayed by a fluorometric method with the exception of 5-hydroxytryptamine in which a radioenzymatic procedure was utilised.

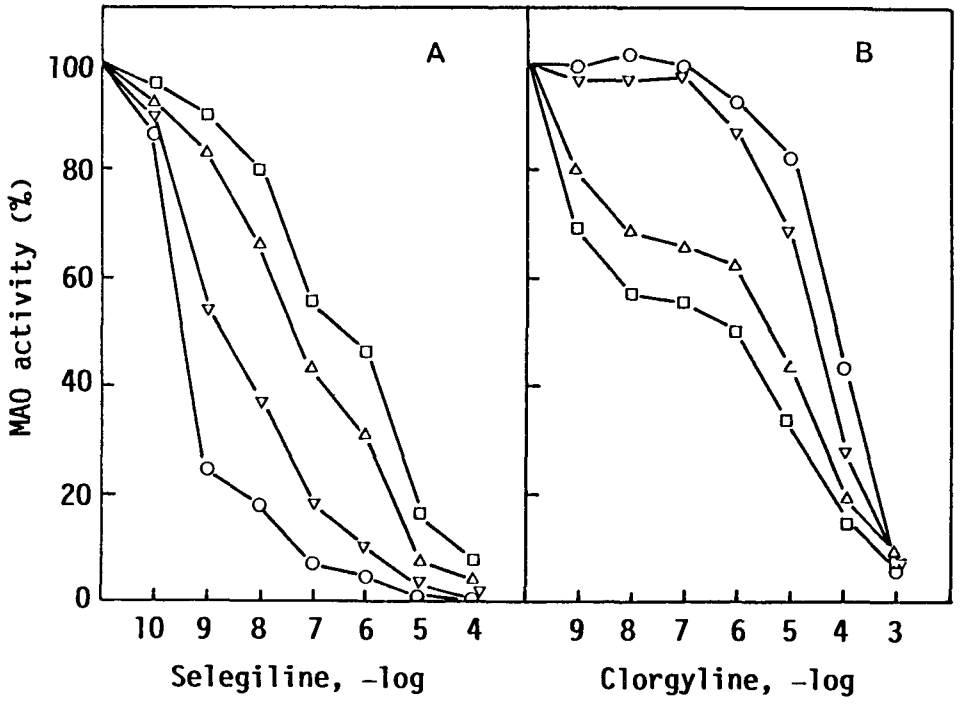

FIG. 2. Inhibition of deamination of n-hexylamine of different concentrations by various concentrations of: Panel A, clorgyline and Panel B, selegiline. The experiments were carried out as described in the legend to Fig. 1. The concentrations of n-hexylamine were: 2×10^{-3} M (□), 5×10^{-4} M (△), 1×10^{-4} M (▽) and $2{\cdot}5 \times 10^{-5}$ M (○).

neither is it known which type of MAO is involved in the catabolism. Before a systematic kinetic analysis of the deamination of the above aliphatic amines was made, initial experiments were conducted to show that the reactions were linear for at least 15 min with increasing enzyme concentrations. It was confirmed in the present experiments that during prolonged incubation times, the enzyme activity was inhibited (von Korff & Wolfe 1984). Therefore, a 10 min incubation time period was chosen for all the experiments described below.

Rat liver mitochondrial MAO was preincubated at room temperature (20°C) at pH 7·4 for 30 min with either the selective MAO-A inhibitor clorgyline (5×10^{-7} M) or the MAO-B inhibitor selegiline (1×10^{-6} M) before measuring the remaining MAO activity towards n-butylamine, n-hexylamine, n-heptaylamine, n-decylamine and n-dodecylamine along with β-phenylethylamine and 5-hydroxytryptamine as substrates as shown in Fig. 1A. MAO is extremely sensitive towards selegiline with respect to the deamination of aliphatic amines. The shorter chain aliphatic amines appear to be relatively more sensitive than the longer chain amines and even more sensitive than the typical MAO-B substrate β-phenylethylamine. n-Butylamine deamination, for instance, was inhibited by over 90% at a concentration of 10^{-8} M selegiline. This concentration caused only a 50% inhibition with respect to the deamination of β-phenylethylamine. 5-Hydroxytryptamine is a typical MAO-A substrate; its deamination, as expected, is insensitive towards selegiline. Such results indicate clearly that these aliphatic amines are metabolized preferentially by MAO-B. This has been further confirmed by observing that, after inhibition by clorgyline, MAO activity towards the aliphatic amines was much greater that it was towards the typical MAO-A substrate 5-hydroxytryptamine (Fig. 1B). It is unclear why the deamination of longer chain aliphatic amines (n-decylamine and n-dodecylamine) is not more sensitive towards clorgyline than it is for the shorter chain amines (n-butylamine).

Table 1. Kinetic parameters of rat liver MAO-B with respect to aliphatic amines and diamines of different chain length.

	V_{max} (nmol min^{-1} mg^{-1})	K_m (1×10^{-4} M)	V_{max}/K_m
Methylamine	0*	—	—
Ethylamine	0*	—	—
n-Propylamine	3·49 ± 0·06	51·27 ± 0·22	0·07
n-Butylamine	6·18 ± 0·16	1·21 ± 0·10	5·11
n-Pentylamine	5·69 ± 0·26	0·15 ± 0·02	37·40
n-Hexylamine	7·87 ± 0·37	0·17 ± 0·03	46·29
n-Heptylamine	6·74 ± 0·77	0·13 ± 0·02	51·85
n-Octylamine	8·65 ± 0·62	0·14 ± 0·03	61·80
n-Nonylamine	6·78 ± 0·23	0·12 ± 0·02	56·50
n-Decylamine	7·01 ± 0·20	0·18 ± 0·02	38·94
n-Dodecylamine**	5·11 ± 0·82	5·50 ± 1·36	0·93
n-Octadecylamine**	0·62 ± 0·08	12·21 ± 3·29	0·05
Putrescine	0*	—	—
Cadaverine	0*	—	—
Amagtine	0*	—	—
β-Phenylethylamine	7·89 ± 0·38	0·08 ± 0·01	98·63

* No enzyme activity was detected.
** The substrates were not totally soluble in the incubation mixture. A homogenous suspension of the substrate was obtained by ultrasonication.

It has been previously observed that the extent of inhibition of MAO activity by selective MAO inhibitors depends on the substrate concentrations used in the assays. The type B properties of β-phenylethylamine disappeared, for example, with increasing substrate concentration (Suzuki et al 1979; Kinemuchi et al 1980). The inhibition patterns of the deamination of n-hexylamine, for example, at different substrate concentrations by selegiline and clorgyline are illustrated in Fig. 2. As with β-phenylethylamine as substrate, n-hexylamine exhibited high sensitivity towards selegiline and low sensitivity towards clorgyline. Clearly, the V_{max} values of the enzyme decreased and K_m

values increased with the increase of MAO-A/MAO-B proportions. With increasing substrate concentrations, biphasic inhibitor responsive curves were observed, indicating that the n-hexylamine was metabolized by MAO-A at high concentration. For instance, n-hexylamine at $2{\cdot}5 \times 10^{-5}$ M, more than 90% deamination was catabolized by MAO-B. When the substrate concentration was increased to 2×10^{-3} M, approximately 50% of the deamination was due to MAO-A. This was also the case for other aliphatic amines at very high substrate concentrations (results not shown).

In Table 1, a systematic comparison of the kinetic parameters of MAO-B with respect to unbranched aliphatic amines containing from one to 18 carbons is listed. Methylamine and β-ethylamine at concentrations up to 2×10^{-2} M were not metabolized by either MAO-B or MAO-A. The metabolism of methylamine and ethylamine found in-vivo by Rechenberger (1940) is probably not catalysed by MAO. n-Propylamine and n-butylamine can be deaminated by MAO-B, but the K_m values are high and the V_{max} values are low. These amines, therefore, are also poor substrates. n-Pentylamine, n-hexylamine, n-heptylamine, n-octylamine, n-nonylamine and n-decylamine are all substrates exhibiting high affinity towards MAO-B. It is surprising that their K_m values are quite low in comparison to the K_m's of most of the endogenous aromatic amine substrates (i.e., all in the 10^{-4} M range). n-Dodecylamine and n-octadecylamine can also be metabolized by MAO-B; their V_{max} values, however, are lower and their K_m values are higher than the C_5–C_{10} aliphatic amines. The increase in chain length reduces the affinity of aliphatic amines towards MAO-B but it is difficult to obtain true kinetic parameters because the long chain substrates become increasingly insoluble in the aqueous incubation medium. V_{max}/K_m values have indicated that as MAO substrate the optimal range of the chain length of the aliphatic amines is from 5 to 10 carbon atoms.

Diamines, such as putrescine, cadaverine, and amagtine were not oxidized by MAO and this confirms the observation using guinea-pig liver enzyme made by Blaschko in 1952.

The kinetic parameters of MAO-A (i.e., enzyme pretreated with 1×10^{-6} M selegiline) with respect to the aliphatic amines were difficult to obtain because of their low affinities toward the enzyme (i.e. since $K_m > 1 \times 10^{-3}$ M, a very high substrate range was required) as a consequence of their limited solubility and because at these higher substrate concentrations the inhibit MAO activity probably on account of non-specific lipophilic interactions between the enzyme and substrates.

The high affinity of aliphatic amines towards MAO-B is probably related to their lipophilicity which is a common property of the typical MAO-B substrates, such as β-phenylethylamine and benzylamine. Such a finding is also consistent with the observation that organic solvents, such as n-hexene and alcohols, at low concentrations selectively inhibit MAO-B activity (Fowler et al 1980; Yu 1984) and that perhaps the selective action of MAO-B is simply due to lipid partition effects of the substrates.

MAO-B exhibits high affinity towards aliphatic amines, which, however, are not present endogenously. It may be involved in the oxidation of these amines from exogenous sources. More interestingly, the present findings suggest that the longer chain aliphatic amine derivatives may be useful in the design of certain prodrugs, because of increased lipophilicity.

I thank Dr A.A. Boulton for advice; Saskatchewan Health and the Medical Research Council of Canada for their continuing financial support; D. Young for technical assistance.

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