Studies of Monoamine Oxidase and Semicarbazide-Sensitive Amine Oxidase

I. Inhibition by a Selective Monoamine Oxidase-B Inhibitor, MD 780236

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Abstract—In vitro studies of the effect of MD 780236, a selective monoamine oxidase (MAO)-B inhibitor, on a semicarbazide-sensitive amine oxidase (SSAO) in rat testis and lung showed that this compound dose-dependently inhibited SSAO activity. The extents of inhibition of MAO-A, -B and SSAO in these two rat tissues by this compound after 30 min of preincubation were found to be MAO-B > MAO-A > SSAO. This selectivity was also evident in preparations without preincubation. Degree of inhibition of SSAO was not significantly influenced by pretreatment with either 10^{-3} M clorgyline, I-deprenyl or 10^{-4} M SKF 525A. Inhibition of SSAO was not enhanced by varying the time of preincubation of the enzyme and the compound, indicating direct action on and reversible inhibition of SSAO. The inhibition of SSAO by MD 780236 was non-competitive with or without preincubation, with a K_i value of 110 \mu M. Although MD 780236 is a selective and “suicide substrate” inhibitor of MAO-B, these present results indicate that this compound may also inhibit SSAO activity, but by a mechanism different from that for MAO-B. These findings confirm an earlier hypothesis that compounds that inhibit both MAO and SSAO have totally different modes of action on these two different amine oxidases.

Monoamine oxidase [MAO, monoamine:oxygen oxidoreductase (deaminating) (flavin-containing) EC 1.4.3.4] is divided into two forms, MAO-A and -B, on the basis of different substrate specificities and different sensitivities to inhibition by the acetylenic MAO inhibitors, clorgyline and I-deprenyl. MAO-A selectively oxidizes 5-hydroxytryptamine (5-HT) and norepinephrine and is more sensitive to inhibition by clorgyline than by I-deprenyl, while MAO-B selectively oxidizes \( \beta \)-phenylethylamine (PEA) and benzylamine (BZ) and is more sensitive to I-deprenyl than by clorgyline (1–3, also see review 4).

Although little information is available on its physiological role, reports have been accumulating (see, e.g., 5–9) that describe the presence of another amine oxidase, termed semicarbazide-sensitive amine oxidase (SSAO), benzylamine oxidase or clorgyline-resistant amine oxidase, in various tissues of many species. This enzyme differs from MAO and deaminates various monoamines. As so far examined, BZ is the substrate most rapidly deaminated, and this activity is resistant to acetylenic MAO inhibitors at concentrations sufficient to completely inhibit both forms of MAO activity (see review 10). However, this enzyme is highly sensitive to carbonyl reagents such as semicarbazide. In this work, SSAO is the name used for the BZ oxidizing activity that remains after pretreatment with the acetylenic MAO inhibitor, I-deprenyl (10^{-3} M) or clorgyline. In addition to carbonyl reagents, a variety of compounds inhibit this SSAO activity. The inhibitory properties of some
selective MAO inhibitors may differ markedly from their properties as SSAO inhibitors (10). We recently reported the presence of both MAO and SSAO activity in rat testis (11) and lung (12), but the physiological role of SSAO in these tissues is not yet clear.

MD 780236 is a selective and potent MAO-B inhibitor both in vivo (13) and in vitro (14). Several studies have indicated that another selective MAO-B inhibitor, I-deprenyl, potentiates the anti-akinetic effect of 1-DOPA in patients with Parkinson's disease (see review 15). If MD 780236 has a possible use as an adjunct to the treatment of Parkinson's disease, inhibition of enzymes other than MAO will be not negligible for such treatment. We report here the results of comparative studies of inhibition of SSAO and MAO in rat testis and lung by MD 780236. A preliminary report of this work has been published elsewhere (16).

Materials and Methods

Chemicals: The compound, MD 780236, (3-[(3-chlorophenyl)methoxy]phenyl)-5-[(methylamino)methyl] -2-oxazolidinone) methanesulphonate, was kindly provided by the Centre de Recherche Delalande, France. Clorgyline hydrochloride and I-deprenyl hydrochloride by May & Baker Ltd., Dagenham, U.K. and by Dr. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary, respectively. [14C] 5-hydroxytryptamine binoxalate (5-HT, 44 mCi/mmol) and [14C]β-phenylethylamine hydrochloride (PEA, 48.25 mCi/mmol) were purchased from New England Nuclear, Boston, U.S.A. and [14C]-benzylamine hydrochloride (BZ, 56 mCi/mmol) from Radiochemical Centre, Amersham, U.K. Other chemicals used were of the highest grade commercially available.

Enzyme preparation and assay of enzyme activity: Male Wistar rats, weighing about 200 g, were sacrificed and their testes and lungs rapidly removed. Then 10% (w/v) homogenates of these tissues were prepared in 0.25 M sucrose with 10 mM phosphate buffer, pH 7.8, and centrifuged at 600xg for 10 min to remove nuclei and cell debris. The resulting supernatants were used as homogenates in this study. SSAO activity was determined radiochemically at 37°C and pH 7.8 for 10 min for rat lung homogenates and for 20 min for testis homogenates, by the method for MAO (17, 18) with [14C]BZ (10 μM) as the substrate. In inhibition studies, enzyme preparations were preincubated with various concentrations of MD 780236 for 30 min before adding [14C]BZ for assay of the remaining SSAO activity. For some experiments, either 5-HT (MAO-A) or PEA (MAO-B) deamination in these preparations was determined radiochemically, as described above, with [14C]-5-HT (100 μM) or [14C]-PEA (10 μM) as the substrate. In all cases, product formation was linear with the protein and with the time periods of incubation used. Protein concentrations were determined by the modified biuret method (19) with bovine serum albumin as the standard.

Results

The extent of participation of MAO in total BZ deamination, at the assay concentration (10 μM) used for SSAO, was determined by preincubating rat testis and lung homogenates with 10⁻³ M clorgyline or I-deprenyl at 37°C for 30 min to completely inhibit both MAO-A and -B activity. Pretreatment with either MAO inhibitor gave similar results indicating that MAO in both preparations contributed very little (at most, 6%) to the total deamination. Two other substrates used in this study, 5-HT and PEA, are substrates not only for MAO, but also for SSAO (10). The participation of SSAO in the total deaminations of 5-HT and PEA in rat testis and lung, as reported earlier (11, 20), was relatively small (e.g., about 0.3 and 20%, respectively, in the testis) compared to more than 90% for BZ (10 μM). These results indicate that in contrast to the predominant deamination of BZ by SSAO, 5-HT or PEA deamination, at the assay concentrations used, might be predominantly catalyzed by MAO-A (5-HT) or -B (PEA) alone under the conditions used (4). Thus in further experiments, unless otherwise specified, no pretreatment for inhibition of either MAO or SSAO was performed to assay enzyme activity. Another preliminary study showed that MD 780236 did not affect the extraction of deaminated metabolites in the assay used.

Figures 1A and B show, as examples, the
inhibitory effects of preincubation at 37°C for 30 min with various concentrations of MD 780236 on testis and lung SSAO activities. Consistent with the preliminary results (16), preincubation with this compound resulted in dose-dependent inhibition of SSAO activity; inhibition curves for the activity in testis and lung were similar.

To investigate the selective inhibition of testis and lung MAO-A, -B and SSAO by MD 780236, homogenates were preincubated with this compound for 30 min at 37°C, and the remaining activities of MAO-A and -B were then determined. These results were compared with the extent of inhibition of SSAO. Results shown in Fig. 1A and B indicate that this compound is a MAO inhibitor with higher selectivity towards MAO-B than -A in these two preparations (IC50 for MAO-B/IC50 for -A ratio was about 1:16 for the lung and 1:25 for the testis), under the conditions used. As is also shown, although this compound was a weaker inhibitor of MAO-A, the extent of inhibition of 5-HT deamination (MAO-A) was found to be greater than that of SSAO. Data are not shown here, but this MAO-B selectivity was also evident without preincubation of the two enzyme preparations with MD 780236.

In subsequent experiments, we compared the extent of inhibition of SSAO before and after complete inhibition of both forms of MAO by pretreatment with clorgyline (10^-3 M) (Figs. 1A and B). Preincubation with MD 780236 for 30 min caused similar inhibition of SSAO in both rat homogenates with and without clorgyline-pretreatment. This was also true for preparations pretreated with l-deprenyl (10^-3 M) (data not shown). In similar experiments, pretreatment with an inhibitor of microsomal monooxygenases, SKF 525A (10^-4 M) also did not significantly change the extent of inhibition (data not shown). This was also true for rat lung microsomal fractions that had previously been shown to contain both MAO and SSAO activities, even though the MAO activity might be due to contamination from mitochondrial membrane fragments (12).

Study of the time-course of inhibition of SSAO by MD 780236 indicated that extents of inhibition did not change even for pre-
incubation time up to 90 min at 37°C (Table 1). This may indicate that this compound is a reversible inhibitor of SSAO.

The mode of inhibition of rat lung SSAO by MD 780236 was determined from Lineweaver-Burk plots with different BZ concentrations (Fig. 2). This compound inhibited SSAO activity non-competitively with approximate Kᵢ values determined from linear slope replots of 110 μM, without and with preincubation at 37°C for 30 min. Results obtained without preincubation are shown in Fig. 2. This Kᵢ value for the inhibition of SSAO is in line with the value of 81 μM reported for rat aorta SSAO (21).

### Discussion

Although its physiological role is not yet clear, SSAO in certain rat cardiovascular tissues appears to deaminate various monoamines such as BZ, PEA, kynuramine and dopamine (10). This enzyme in other rat tissues, including lung, deaminates other monoamines, including tryptamine, n-pentylamine and tyramine to different extents (10). As described above, BZ is the best substrate so far examined for this enzyme. In the present work, comparison of data with and without the MAO inhibitor pretreatment indicated BZ deamination as being predominantly by SSAO at the concentration used (10 μM). This finding is consistent with the result of about 85–90% of the total BZ deamination deriving from rat heart SSAO, reported by Clark et al. (22). It seems likely that this large contribution of SSAO to the total BZ deamination at the assay concen-

### Table 1. Effect of preincubation time with MD 780236 on semicarbazide-sensitive amine oxidase in rat lung

| Preincubation time (min) | Inhibition of SSAO activity (%) caused by MD 780236 at: |
|-------------------------|---------------------------------------------------|
|                         | 0.1 μM     | 50 μM     | 500 μM    |
| 0                       | 15.3±1.8  | 47.2±1.5  | 79.5±0.2  |
| 20                      | 19.9±0.8  | 50.1±0.8  | 81.0±0.4  |
| 40                      | 22.9±3.9  | 52.3±1.2  | 78.5±0.2  |
| 90                      | 17.6±0.6  | 48.5±2.2  | 73.7±1.3  |

Rat lung homogenate was preincubated with indicated concentrations of MD 780236 at 37°C for the indicated times, and then remaining SSAO activity was assayed, as described in the text. Values were expressed as percent inhibition of SSAO activity in preparations preincubated with water for the same periods. Values are means±S.E. (n=4).

![Double reciprocal plot](image-url)
tration may be mainly due to the difference in the \( K_m \) values of SSAO (around 5 \( \mu \)M, 10, 11) and MAO-B (around 160 \( \mu \)M, 10, 23) for this substrate. Consequently, low BZ concentrations can be used for studying BZ deamination by SSAO alone in tissues, even though MAO may be present (10). This will also be true for 5-HT or PEA, used for assaying either MAO-A or -B activity. As described in the text, deamination of both substrates by SSAO was evident, but the degree of the deamination was extremely small compared to that of BZ. In addition, our experiments confirmed earlier results (14) that MD 780236 selectively inhibited MAO-B more than -A. This indicates that the small contribution of SSAO to deamination of 5-HT and PEA did not interfere with the conclusions derived from the present experiments.

MD 780236 is a selective MAO-B inhibitor, and in vitro, this compound inhibits this MAO by acting as a preincubation-time-dependent, “suicide substrate” inhibitor (14). Such inhibition involves an initial non-covalent and reversible interaction between the enzyme and MD 780236. The product of an intermediate complex of the enzyme and the compound then either breaks down into products or reacts with the enzyme to form a stable covalently-bound adduct (14). With MAO-A, the role of MD 780236 as a substrate predominates with virtually no inhibition (24), and it is transformed into metabolites both in vivo (24) and in vitro (14). One of these metabolites (the alcoholic derivative) shows a higher selectivity for inhibition of MAO-B in vitro than its parent compound, MD 780236 (14). Rat testis contains predominantly, but not exclusively, MAO-A activity (25), whereas rat lung predominantly contains MAO-B activity (12). In the present study, to investigate whether inhibition of SSAO might be due to a metabolite(s) from MD 780236 by MAO-A in the testis or by -B in the lung, we compared the extent of inhibition of SSAO before and after clorgyline- or l-deprenyl-pretreatment. Results showed similar inhibition of SSAO, suggesting that metabolite(s) presumably formed by MAO-A or -B did not affect the extent of SSAO inhibition. This was also confirmed by experiments with SKF 525A. This lack of participation of MAO and monoxygenases systems indicates that MD 780236, as well as being a selective and indirect suicide inhibitor of MAO-B in vitro, may directly inhibit SSAO activity. In addition to these findings, MD 780236 is a reversible inhibitor of SSAO and the mode of inhibition is non-competitive; this is compared to its competitive inhibition of both MAO-A and -B in the initial reversible phase of inhibition (14). All results presented strongly indicate that the mechanism for inhibition of SSAO by this compound in vitro is certainly different from that of MAO-B. This reinforces the hypothesis that inhibitory properties of some MAO inhibitors against MAO activity may differ from their properties as SSAO inhibitors (10, 26, 27).

The \( K_i \) value (110 \( \mu \)M) of reversible inhibition of SSAO by MD 780236 is much higher than that for inhibition of MAO-B in the previous study (0.54 \( \mu \)M), in the reversible phase without preincubation (14). This suggests that pharmacologically-relevant doses of this compound for inhibition of MAO-B should not greatly inhibit SSAO activity in vivo.

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