Studies on 5-Fluoro-α-Methyltryptamine and p-Chloro-β-Methylphenethylamine: Determination of the MAO-A or MAO-B Selective Inhibition In Vitro

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Abstract—To further clarify highly MAO-A- or -B-selective inhibitory properties of 5-fluoro-α-methyltryptamine (5-FMT) and p-chloro-β-methylphenethylamine (p-CMPEA), we determined the types and Kᵢ values of inhibition of rat brain MAO-A and -B activity in vitro. The kinetic data obtained showed that 5-FMT is a competitive MAO-A-selective inhibitor with about a 18,000-fold higher sensitivity than MAO-B. In contrast, p-CMPEA is a competitive MAO-B-selective inhibitor with about a 620-fold higher sensitivity. Based on the present findings of highly MAO-A- or -B-selective inhibition, these two compounds might prove to be of value in in vivo studies.

The mitochondrial enzyme monoamine oxidase (MAO, EC 1.4.3.4) catalyzes the oxidative deamination of various monoamines, and it exists in two forms, termed form A (MAO-A) and form B (MAO-B) (for review, see ref. 1). There are now a large number of MAO inhibitors selective for either MAO-A or MAO-B. Some of the reversible MAO-A-selective inhibitors are simple α-methylated substrate-analogues (2–5), but many of the other compounds are reported to be reversible MAO-B-selective inhibitors. From the experimental findings, MAO-A-selective inhibitors are clinically effective antidepressants, but an irreversible MAO-B-selective inhibitor, l-deprenyl, potentiates the anti-akinetic effect of L-DOPA in patients with Parkinson's disease, probably by preventing the degradation of dopamine thus formed from the parent compound (6). Very few useful reversible MAO-B-selective inhibitors have been reported in the literature, and even in these cases, the selectivity was rather small compared to that of reversible MAO-A-selective inhibitors (6).

In our preliminary work (7), we found that halogenated α- or β-methylated substrate-analogues such as 5-fluoro-α-methyltryptamine (5-FMT) and p-chloro-β-methylphenethylamine (p-CMPEA) are reversible MAO-A- and MAO-B-selective inhibitors, respectively. This conclusion was derived from data on their IC₅₀ values for inhibition of both forms of MAO and complete recoveries of the enzyme activity after dilution of enzyme preparations treated with either compound. In the present study, for further in vivo studies, the types of inhibition and the Kᵢ values of rat brain MAO-A and -B by these two analogues were determined. Although the IC₅₀ values obtained earlier (7) indicate the selective inhibition of either form of MAO, they will greatly change depending on the substrate concentrations used for determining the values, if these analogues competitively inhibit MAO activity. A preliminary report of this work has been published elsewhere (8). The homogenates of Male Sprague-Dawley rat forebrain were prepared in 0.32 M sucrose with 10 mM phosphate buffer (pH 7.4) and used as the enzyme sources. MAO-A or -B activity was assayed radiochemically as described previously with 0.1 mM [¹⁴C]5-hydroxytryptamine (5-HT) or 0.1 mM...
[¹⁴C]benzylamine (BZ) as the respective substrate at 37°C (9). 5-FMT HCl and p-CMPEA HCl were obtained from Sigma Chemical Co., Ltd.

In confirming the earlier result (7), oxidation of 5-HT (MAO-A) was inhibited by lower concentrations of 5-FMT than those for inhibition of BZ oxidation (MAO-B). Figure 1, as an example, shows such results analyzed using double reciprocal plots. The plots indicate that 5-FMT is a competitive inhibitor of both MAO-A and -B. Data are not shown here, but similar analyses showed that p-CMPEA is also a competitive inhibitor of both forms of MAO with a high MAO-B selectivity. Table 1 summarizes all the data obtained in the present study. A ratio of the K_i values of 5-FMT for the inhibition of MAO-A and -B represents the definitely high MAO-A selectivity, whereas the ratio of p-CMPEA represents an approximately 620-fold MAO-B selectivity. These ratios confirm the previous results (7) that 5-FMT is a highly MAO-A-selective inhibitor, whereas p-CMPEA is a highly MAO-B-selective inhibitor. The result obtained with the former compound is compatible with earlier findings that α-methylated substrate-analogues are reversible MAO-A-selective inhibitors (2–5).

Since MAO-A and MAO-B classification has established, inhibitors selective for MAO-A or -B have been developed which have opened new possibilities for selective treatment. It was, however, previously suggested that development of reversible, high MAO-B-selective inhibitors is probably difficult, since active centers of MAO-A and -B are only partly different and a reversible MAO-A-selective inhibitor can bind to MAO-A, but the inhibitor is sterically hindered from binding to the active center of -B (6). On the other hand, a selective MAO-B inhibitor is not sterically hindered from binding to the active center of MAO-A. All data presented in this

![Fig. 1. Double reciprocal plots of inhibition of MAO-A and -B by 5-FMT.](image)

**Table 1.** Selectivity for inhibition of MAO-A and -B by 5-FMT and p-CMPEA

<table>
<thead>
<tr>
<th>Ki value (µM) for</th>
<th>MAO-A</th>
<th>MAO-B</th>
<th>Selectivity</th>
<th>-fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FMT</td>
<td>0.032</td>
<td>575</td>
<td>MAO-A</td>
<td>17,969</td>
</tr>
<tr>
<td>p-CMPEA</td>
<td>340</td>
<td>0.55</td>
<td>MAO-B</td>
<td>618</td>
</tr>
</tbody>
</table>
series clearly show that the two analogues tested here had extremely high selectivity towards MAO-A or -B in a reversible manner. Thus these two analogues might be of value for in vivo studies to determine whether or not either of these two compounds is also a selective inhibitor of MAO-A or -B with such a high selectivity.

References