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β-Phenylethanolamine as a Substrate for Monoamine Oxidase

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Monoamine oxidase (amine–oxygen oxidoreductase (deaminating) (flavin containing), EC 1.4.3.4) is thought to exist in at least two forms, A and B, which differ in substrate specificity and inhibitor-sensitivity. Houssay & Tipton (1974) have suggested structural criteria for predicting the substrate specificity of each form of the enzyme from rat liver. For phenethylamine derivatives these are: (a) type-A substrates must carry a p-hydroxyl group; (b) type-B substrates must be devoid of a p-hydroxyl group in the side chain. Amines fulfilling both these conditions are substrates for both enzymes. This classification appears to preclude β-phenylethanolamine, which satisfies neither criterion, although there are reports of oxidation of this amine by monoamine oxidase (e.g. Pratesi & Blaschko, 1959). The use of whole-tissue preparations in some studies allows the possibility of oxidation by enzymes other than the mitochondrial monoamine oxidase. The oxidation of β-phenylethanolamine and its enantiomers by a mitochondrial preparation from rat liver has therefore been re-investigated.

Mitochondria were prepared by the method described by Hawkins (1952) and finally suspended in phosphate buffer, pH 7.4, prepared by mixing 0.05M solutions of KH₂PO₄ and Na₂HPO₄. D(-)- and L(+)-β-Phenylethanolamines were prepared from the corresponding mandelic acids. The purified hydrochlorides had optical rotations, [α]D⁰² of -44.9 ± 1.6 and +46.9 ± 1.4, measured in water. Monoamine oxidase activity was measured by following O₂ uptake in an oxygen electrode at 30°C and at pH 7.4 (Williams, 1974). The reaction was started by adding substrate in 0.05ml of the above-mentioned buffer to an incubation medium containing KCN (1 mM), mitochondrial protein (6mg), in 1.95ml of the same buffer. Preliminary studies showed that maximum velocities were obtained at phenylethanolamine concentrations of 3 mM. This concentration was used in all experiments. Oxidation of the amine was studied both in the presence and absence of the selective inhibitors N-3-(2,4-dichlorophenoxy)propyl-N-methylprop-2-ynylamine (Clorgyline) and N-(2-phenylisopropyl)-N-methylprop-2-ynylamine (Deprenil). Results are shown in Table 1.

It is clear that the amine is a substrate for monoamine oxidase, although rates of oxidation are slow compared with those obtained with substrates such as tyramine or benzylamine. Under comparable conditions the latter amines were turned over at rates 3–4 times those shown in Table 1. The D(-)-isomer of β-phenylethanolamine is oxidized more readily than the L(+)-form. The use of Clorgyline and Deprenil produced noteworthy results. At concentrations of the former that produced 50% inhibition of monoamine oxidase A, but which left the B enzyme unaffected, the rate of oxidation of the D(-)-isomer was halved, whereas the L(+)-isomer was barely affected. When Deprenil
Table 1. *Oxidation of isomers of \( \beta \)-phenylethanolamine by mitochondrial monoamine oxidase*

Enzyme activity was measured by measuring O\(_2\) uptake at pH 7.4 and at 30°C. Substrate concentrations were 3 mM.

<table>
<thead>
<tr>
<th>( \beta )-Phenylethanolamine isomer</th>
<th>No inhibitor</th>
<th>Clorgyline (100 nM)</th>
<th>Deprenil (1 ( \mu )M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(+)</td>
<td>36.5</td>
<td>35.5</td>
<td>17</td>
</tr>
<tr>
<td>D(−)</td>
<td>75</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>DL</td>
<td>48</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

was similarly used to block the B enzyme, a similar decrease in the rate of oxidation of the L(+) isomer was observed, with a lesser effect on the other optical isomer. This suggests that monoamine oxidase A acts only on d(−)-\( \beta \)-phenylethanolamine, whereas the B enzyme will oxidize both d(−)- and L(+) forms. This apparent optical specificity is particularly noteworthy because the d(−)-isomer has the same configuration as the naturally occurring laevorotatory form of noradrenaline, itself a substrate for monoamine oxidase. It is not clear from previous reports whether other phenylethanolamines such as adrenaline, noradrenaline and their O-methyl derivatives show the same behaviour, since most studies of the optical specificity of monoamine oxidase were carried out before the multiple nature of the enzyme was established. There are reports that (−)-noradrenaline is oxidized more readily than is the d-form by monoamine oxidase from several different tissues (Blaschko et al., 1937; Pratesi & Blaschko, 1959; Giachetti & Shore, 1966). These findings agree with those of the present study, where the absolute specificity of monoamine oxidase became apparent only when the B enzyme was partly inhibited.

The present findings do not reveal why \( \beta \)-phenylethanolamine does not conform to the specificity criteria outlined earlier, i.e. why it acts as a substrate at all. In delineating the specificities of monoamine oxidases A and B, Houslay & Tipton (1974) pointed out the anomalous behaviour of 5-hydroxytryptamine. It now appears that \( \beta \)-phenylethanolamine also fails to fit into the general pattern that they described.


A Sensitive Quantitative Assay Method for Dolichols, Cholesterol and Ubiquinone using High-Pressure Liquid Chromatography

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There is currently no satisfactory sensitive assay for polyisoprenoid alcohols. In the past these compounds have been assayed either by weighing purified samples or by spot size on t.i.c. (e.g. Butterworth & Hemming, 1968). Donnahey & Hemming (1975) described an assay procedure based on high-pressure liquid chromatography in which a refractive-index detector was used. In this procedure individual ficaprenols could be separated