Amine Oxidases

XIX. INHIBITION OF MONOAMINE OXIDASE BY PHENYLCYCLOPROPYLAMINES AND IPRONIAZID*

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In the eighteenth paper of this series (3), it was shown that monoamine oxidase of beef liver mitochondrial preparations was more strongly and more specifically inactivated by cis- and trans-2-phenylcyclopropylamine than by the most powerful hydrazine derivatives. We continued this investigation and compared the mechanism of action of phenylcyclopropylamines with that of 1-isonicotinyl-2-isopropylhydrazine (iproniazid).

EXPERIMENTAL PROCEDURE

trans-2-Methylcyclopropylamine maleate was secured from J. H. Biel (Lakeside Laboratories), 1-phenylcyclopropylamine hydrochloride from S. C. Bunce (Rensselaer Polytechnic Institute, Troy, New York), cis- and trans-2-phenylcyclopropylamine hydrochloride from H. Green (Smith, Kline, and French), and 1-isonicotinyl-2-isonicotinylphosphate (iproniazid) from M. J. Schiffrin (Hoffmann-La Roche). 4-Phenyl-n-butylamine was prepared by reduction of the corresponding nitrile with LiAlH₄, as described for phenylpropylamine by Fort and Roberts (4). Its hydrochloride, m.p. 162-164°C, was obtained by precipitation from an ethereal solution of the amine and recrystallization from cyclohexane.

\[
\text{CsH}_{10}\text{CIN} \\
\text{Calculated: N 7.55 %} \\
\text{Found: N 7.62 %}
\]

In most cases, 0.01 M tyramine hydrochloride was incubated in 0.067 M phosphate buffer, pH 7.2, at 38°C ("standard conditions") (5-7). As enzyme sources, homogenates prepared from 0.1 g of tissue or the equivalent amounts of mitochondria or solubilized mitochondria served in a system for which the total volume was 2 ml. In general, the inhibitor was incubated with the enzyme for 20 to 30 minutes before the substrate was added. In preparing solubilized mitochondria with the help of octylphenoxypolyethoxyethanol (8), the insoluble material was removed by centrifugation at 20,000 x g for 30 minutes. The \(Q_{OX}\) values, calculated from the initial portion of the curve of oxygen consumption, are defined as the number of microequivalents of oxygen consumed per hour per g of fresh tissue or its equivalent. The \(Q_{AM}\) values represent the production of ammonia in micromoles per hour of incubation with no regard for initial reaction velocities. The \(pI_{50}\) values give the negative logarithms of inhibitor concentrations required to produce 50% inhibition of the initial oxygen uptake (\(pI_{OX}\)) or of the total ammonia production (\(pI_{AM}\)) (3).

RESULTS

Variation of Length of Preincubation Period—It has already been shown that for maximal inhibition, iproniazid must be incubated with mitochondrial and solubilized monoamine oxidase for 6 to 12 minutes before addition of the substrate (5, 8). Similar results were obtained with trans-2-PCP which caused maximal inhibition after 8 to 12 minutes of initial incubation (Fig. 1).

Influence of Anaerobiosis—It has also been observed that the rat liver enzyme is much less inhibited by iproniazid in the absence of oxygen, than in its presence (8). These results were confirmed and extended for beef liver preparations (Table I). trans-2-PCP, however, did not require oxygen for its inhibitory action and even appeared slightly more efficient when oxygen was omitted during the preincubation period (10).

Effect of Tyramine and Phenylbutylamine on PCP Inhibition—When tyramine was added to the enzyme solution simultaneously with 2 \(\times\) 10⁻⁴ M \(cis-\) or \(trans-2-PCP\), the degree of inhibition dropped with increasing substrate concentration (Table II). No protective action, however, was observed when tyramine was tipped in 15 to 30 minutes after the inhibitors. Approximately one-tenth of the former PCP concentration was now sufficient to produce the same inhibiting response. Furthermore, prolonged dialysis of the enzyme-PCP complex restores only a very small fraction of the tyramine-oxidizing activity (3). It seems, 1

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1 The abbreviations used are: PCP, phenylcyclopropylamine; PBA, 4-phenyl-n-butylamine.
2 Increasing degree of inhibition with increasing preincubation time was also observed for another inhibitor, \(N\)-benzyl-\(N\)-methyl-2-propyramine (9).
3 Barbato and Abood (11) state that inhibition of monoamine oxidase by \(trans-2-PCP\) is not "oxygen-dependent."
undergo rapid oxidative deamination by monoamine oxidase as a substrate. Recently, this compound was found to be used as a substrate. Similar conclusions have been reported closely resembles an irreversible inhibition when tyramine is present in intact and solubilized rabbit liver mitochondria (12).

Therefore, that the effect of 2-PCP on monoamine oxidase very closely resembles an irreversible inhibition when tyramine is used as a substrate. Similar conclusions have been reported for iproniazid (7, 8).

Entirely different data were collected with 4-phenyl-n-butylamine, within a few minutes, was able to reactivate the oxidase, which appeared to be completely inactivated by cis-2-PCP (Fig. 2). Clearly, in the presence of phenylbutylamine, cis- and trans-PCP become weak and reversible inhibitors.4

Effect of Phenylbutylamine on the Inhibitory Action of Iproniazid—Since the replacement of tyramine by phenylbutylamine influenced the inhibition of monoamine oxidase by PCP, similar experiments were carried out with iproniazid. The change of substrates, however, did not affect the p_50 values (Table III) nor did phenylbutylamine reverse the blocking of the system monoamine oxidase-tyramine-iproniazid (Fig. 2). 5

Inhibitory Power of Two Other Cyclopropylamine Derivatives—By a factor of 10^5, 2-PCP is a more effective inhibitor than cyclopropylamine (3). In attempting to breach this enormous gap trans-2-methylcyclopropylamine was tested (Table IV). The inhibitory efficiency of this compound was remarkably higher than that of cyclopropylamine but still inferior to that of 2-PCP. Solubilization of the mitochondria did not alter the outcome of the experiment.

One isomer of 2-PCP, I-phenylcyclopropylamine, displayed a high blocking activity (Table III) which was stronger than that of any other compound devoid of an α-hydrogen atom. The differences in inhibitory power between the 1- and 2-substituted analogues remained essentially constant for various enzyme preparations. Again, after replacing tyramine with phenylbutylamine, the p_50 values dropped sharply for 1-PCP.

DISCUSSION

The data presented in this paper confirm that cis- and trans-2-PCP are strong inhibitors of monoamine oxidase in vitro (3)

4 Since the reaction rate at the concentration of phenylbutylamine used here is depressed as compared with the rates observed with much lower substrate concentrations, the competitive nature of the inhibition by PCP in the presence of phenylbutylamine cannot be expressed in terms of a Lineweaver-Burk diagram (unpublished data).

5 Phenylbutylamine does not reverse the action of 1-isonicotinyl-2-(2-benzylethoxamidoethyly)hydrazine (Nialamid) or N-benzyl-N-methyl-2-propynylamine (unpublished data).

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**TABLE I**

Influence of oxygen on the inhibition of liver monoamine oxidase

One set of manometric vessels was flushed with oxygen and the other set with nitrogen, which had passed through two bottles containing alkaline pyrogallol solution to remove residual oxygen. Then, from one side arm, the inhibitors were tipped in (final concentrations: iproniazid, 10^{-5} M; trans-2-PCP, 10^{-5} M). After 10 minutes, the substrate was added from the second side arm to the mitochondrial suspension, and the nitrogen was replaced by oxygen as rapidly as possible. To increase the protective action of the substrate, its concentration was raised to 0.05 M.

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**TABLE II**

Influence of substrate concentration on inhibition of monoamine oxidase by 2-PCP

The inhibitors were added at the same time or 30 minutes before tyramine to the solubilized mitochondria. Conditions of measurement are those described as “standard.”

![Graph](image-url)
TABLE III
Inhibitory power of phenylcyclopropylamines and iproniazid on liver monoamine oxidase

<table>
<thead>
<tr>
<th>Species and preparation</th>
<th>Substrate</th>
<th>1-PCP</th>
<th>1-pHm</th>
<th>cis-2-PCP</th>
<th>cis-2-pHm</th>
<th>trans-2-PCP</th>
<th>trans-2-pHm</th>
<th>Iproniazid</th>
<th>pI50</th>
<th>pIh0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Tyramine</td>
<td>5.1</td>
<td>4.8</td>
<td>6.8</td>
<td>6.9</td>
<td>7.0</td>
<td>7.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
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<tr>
<td>Intact mitochondria</td>
<td>Tyramine</td>
<td>4.9</td>
<td>4.0</td>
<td>6.8</td>
<td>6.8</td>
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<td>6.8</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
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<tr>
<td>Solubilized mitochondria</td>
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<td>3.8</td>
<td>3.9</td>
<td>5.3</td>
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<td>5.3</td>
<td>5.1</td>
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<tr>
<td>Rabbit</td>
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<td>4.4</td>
<td>4.3</td>
<td>6.0</td>
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<td>5.7</td>
<td>5.5</td>
<td>4.9</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>Intact mitochondria</td>
<td>PBA</td>
<td>4.1</td>
<td>4.3</td>
<td>6.0</td>
<td>6.0</td>
<td>5.7</td>
<td>5.6</td>
<td>5.0</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Solubilized mitochondria</td>
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<td>1.6</td>
<td>3.4</td>
<td>3.4</td>
<td>3.2</td>
<td>2.7</td>
<td>5.6</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>Solubilized mitochondria</td>
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<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Tyramine</td>
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<td>4.3</td>
<td>5.8</td>
<td>5.6</td>
<td>5.5</td>
<td>5.5</td>
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</tbody>
</table>

* Substrate and inhibitor added simultaneously; in all other series inhibitor added first (see "Experimental Procedure").

TABLE IV
Effect of cyclopropylamine and trans-2-methylcyclopropylamine on beef liver enzyme

Since these amines are volatile under the conditions of the Conway test, no ammonia was determined in the presence of these substances. The pI50 values, therefore, were computed from initial rates of oxygen consumption only. The experiments were carried out with mitochondria under conditions described as "standard."

<table>
<thead>
<tr>
<th>Species and preparation</th>
<th>Substrate</th>
<th>Oxygen</th>
<th>Ammonia</th>
<th>Cyclopropylamine</th>
<th>2-Methylcyclopro-&lt;sub&gt;p&lt;/sub&gt;yamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria</td>
<td></td>
<td>176</td>
<td>149</td>
<td>† 2</td>
<td>3.6</td>
</tr>
<tr>
<td>&quot;Solubilized&quot; mitochondria</td>
<td></td>
<td>104</td>
<td>96</td>
<td>1.4†</td>
<td>3.7</td>
</tr>
</tbody>
</table>

* pI50 for rabbit liver mitochondria: 1.5 (1). At 0.01 M concentration, 10% inhibition was observed.
† At 0.04 M concentration, 10% inhibition was observed.

and indicate that a number of parameters markedly influence the degree of inhibition.

The inhibitory power of cyclopropylamine, 2-methylcyclopropylamine, 1-PCP, and 2-PCP increases in that order. Since the electron-releasing methyl residue and the electron-attracting benzene ring both enhance the inhibitory power of cyclopropylamine, although with different intensity, these two substituents probably act by promoting the attachment of the inhibitor to the active site by van der Waals' forces rather than by influencing the reactivity of the amino or cyclopropyl residues. Ethylamine, propylamine, benzylamine, and phenethylamine may be considered as the respective "backbones" of these cyclopropylamine derivatives, and their ability to serve as substrates of the amine oxidase improves in this order (13). Here again the conversion of the smallest unit, ethylamine, to a phenylated derivative produces a dramatic change in reactivity. When the α-methylene group is replaced by the isosteric NH residue, the series of amines is changed into methyl-, ethyl-, phenyl-, and benzylhydrazine. The latter compound is the strongest inhibitor in this series (2, 14).

Although these data point toward a common structural basis of the substrates and for the two classes of monoamine oxidase inhibitors, some distinct differences exist between the blocking mechanisms of iproniazid and PCP. They include (a) reversibility of inhibition induced by phenylbutylamine, (b) oxygen requirement during preincubation of the enzyme with the inhibitor, and (c) the role of the α-hydrogen atom. Hydrazine derivatives that do not possess an α-hydrogen, e.g. N,N-di-
methylhydrazine or N,N-phenylbenzhydrazine display little inhibitory power (15). On the other hand, 1-PCP (Table III) and 1-alkyl-2-phenylcyclopropylamine are powerful inhibitors in spite of their lack of α-hydrogen atoms.

These results are compatible with the assumption that the α-hydrogen is involved in the binding of hydrazines (and substrates) to the active site; this is formally reminiscent of Racker's mechanism of the degradation of glyceraldehyde 3-phosphate. By dehydrogenation a covalent bond may be established between the active site and the inhibitor (or substrate). Subsequently, the substrate moiety is released in deaminated form, presumably because such an enzyme-inhibitor complex is stable. No experimental observations suggest a similar explanation for the action of cyclopropylamines; these substances apparently form a complex with the active site on the strength of secondary forces alone.

According to the data presented in this and in the preceding paper, monoamine oxidases respond strongly and specifically to the active site of the enzyme with trans.2-phenylcyclopropylamine. trans-2-Phenylcyclopropylamine is 10 and 100 times more effective than 1-isonicotinyl-2-isopropylhydrazine (iproniazid) for the enzymes found in rabbit and beef liver mitochondria, respectively. trans-2-Phenylcyclopropylamine is 10 and 100 times more effective than the standard substrate, tyramine, and cis- or trans-2-phenylcyclopropylamine are increas-
ingly potent inhibitors of monoamine oxidase. trans-2-Phenylcyclopropylamine is 10 and 100 times more effective than 1-isonicotinyl-2-isopropylhydrazine (iproniazid) for the enzymes found in rabbit and beef liver mitochondria, respectively.

2. As with iproniazid, the maximum of inhibitory power is reached only after approximately 10 minutes of initial incubation of the enzyme with trans-2-phenylcyclopropylamine.

3. In contrast to iproniazid, trans-2-phenylcyclopropylamine does not require the presence of oxygen during the preincubation period to produce the full blocking effect on monoamine oxidase.

* A. Burger (University of Virginia) synthesized 1-methyl-2-PCP and found it to be an effective inhibitor of monoamine oxidase (private communication).

4. When tyramine, the standard substrate, is replaced by 4-phenyl-n-butylamine, the essentially irreversible action of phenylcyclopropylamines is replaced by a reversible inhibition. In the presence of tyramine cis-2-phenylcyclopropylamine is more inhibitory by a factor of 80 to 300. No similar substrate effects are observed for iproniazid inhibition.

5. The role of the benzene ring on the formation of the enzyme-inhibitor complex is discussed as well as some differences in the role of the α-hydrogen atom in the reaction mechanisms of iproniazid and phenylcyclopropylamines.

Acknowledgment—We wish to express our gratitude to the generous donors of inhibitor substrates.

REFERENCES
Amine Oxidases: XIX. INHIBITION OF MONOAMINE OXIDASE BY PHENYLCYCLOPROPYLAMINES AND IPRONIAZID

E. Albert Zeller, Satyapriya Sarkar and With the assistance of Renate M. Reinen