Amine Oxidases

XVII. MODE OF ACTION OF 1-ISONICOTINYL-2-ISOPROPYLHYDRAZINE ON MONOAMINE OXIDASE*†

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Iproniazid was found to be an efficient inhibitor of monoamine oxidase (2, 3). By systematically varying the structure of this compound, we have attempted to discover the configuration responsible for its activity and to gain information concerning the nature of the reactive site of monoamine oxidase.

EXPERIMENTAL

Mitochondrial preparations which served as the source of MO† were isolated as described previously (3). Most of the hydrazine derivatives were obtained from Hoffmann-La Roche‡ and were used either as free bases or as hydrochlorides. Symmetric triaminoguanidine (4), 1,2-dibenzoylhydrazine, 1,2-dimethyl-1,2-dibenzoylhydrazine, 1-benzoyl-2-ethylhydrazine, and 1-acetyl-1-ethylhydrazine were prepared in this laboratory by conventional methods.

The assay for MO activity with 0.01 M tyramine has been described in a preceding paper (3). Inhibitors were preincubated with the mitochondrial suspensions for 15 minutes before adding the substrate (3, 5). The \( p_{50} \) values, which represent the negative logarithms of the inhibitor concentration required to produce 50 per cent inhibition of the initial oxygen uptake \( (p_{50}) \) or total ammonia production \( (p_{T}) \) were established graphically from determinations of the concentration range producing less than total inactivation of the enzyme. The symbol \( p_{50} \) < \( p_{T} \) indicates that, at the inhibitor concentration \( a \), inhibition was found to range between 15 and 45 per cent, and the symbol \( p_{50} \) = \( p_{T} \) stands for an inhibition of less than 15 per cent.

The \( Q_{m} \), calculated from the initial portion of the curve of oxygen consumed was defined as the number of microatoms of oxygen consumed per hour per gram of fresh tissue or its equivalent. The \( Q_{m} \) values represent the production of ammonia in micromoles per hour of incubation with no regard to initial reaction velocities. At the end of the incubation period, the number of microatoms of oxygen consumed and the number of micromoles of ammonia produced are determined. The quotient \( O/N \) stands for the ratio of these two figures.

RESULTS

Influence of Alkyl Residues on Inhibitory Power—The results summarized in Table I indicate that neither hydrazine nor acylhydrazides are potent inhibitors of MO. Alkyl derivatives of hydrazine and of acylhydrazides, however, act as efficient blocking agents. The lack of inhibitory activity of hydrazine and acylhydrazides cannot be ascribed to an inability of these compounds to penetrate into the mitochondria. Solubilized MO (3, 0) remains as resistant as mitochondrial MO, whereas the diamine oxidase of rabbit liver mitochondria is strongly inhibited by hydrazine and acylhydrazides.

Effect of Alkyl and \( \alpha \)-Methylalkyl Derivatives of Isonicotin acid Hydrazine on MO—Monosubstitution with alkyl groups on the second nitrogen atom of isonicotinic acid hydrazide produced highly active inhibitors of MO (Table II). Although 1-isonicotinyl-2-methylhydrazine was less efficient than iproniazid, an increase in chain length of the alkyl group was accompanied by an increase in the degree of enzyme inhibition which reached a maximum with the butyl derivative and then declined with the hexyl and heptyl derivatives. The \( p_{50} \) values for mitochondrial MO of beef and mouse liver run closely parallel in this respect. \( \alpha \)-Methylation of the alkyl group (isopropyl, isobutyl, and iso-octyl derivatives) generally reduced the potency of the inhibitor below that of the corresponding straight chain compound.

Effect of 2-Alkylpicolinic Acid Hydrazides on MO—The alkyl derivatives of picolinic acid hydrazide present a slightly different finding from that with the isonicotinic acid hydrazides in that there is no increase in inhibitory power with an increase in the number of carbon atoms in the alkyl substituent. The methyl, ethyl, and isopropyl picolinic acid hydrazides are equally effective as inhibitors of mitochondrial MO (Table III).

Effect of 2,2-Disubstituted Isonicotin Acid Hydrazides on MO—All of the 2,2-dialkyl derivatives of isoniazid (2,2-dimethyl-1, 2,2-trimethyl-, 2,2-diethyl-, 2,2-diisopropyl-INH) and of 1-(2-methylisonicotinic acid) hydrazide (2,2-dimethyl-, 2,2-diethyl-, 2,2-diisopropyl-, and 2,2-diallyl-MeINH) that were tested were practically without effect on mitochondrial MO of mouse and beef liver (\( p_{50} \) = 3; reaction conditions were as described in Table II). The same was true for the 2-alkylidene derivatives of INH, viz. ethyldiene-, propyldiene-, isopropylidene-, isobutyldiene-, cyclohexylidene-, and benzylidene-INH, and for the isonicotinic acid hydrazones of pyruvic acid and

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† For Paper XVI of this series, see Ref. 1.
‡ The abbreviations used are: MO, monoamine oxidase; and IAH, isonicotinic acid hydrazide.
§ We are indebted to the Lilly Research Laboratories, Indianapolis, Indiana, and to Dr. Neal Artz, Corn Products Refining Company, Argo, Illinois, for these compounds.
Effect of alkylated hydrazine derivatives on monoamine oxidase

Beef liver mitochondria were used, with standard conditions. See text.

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Nonalkylated compounds</th>
<th>N-2-alkylated compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( p_{\text{ox}} )</td>
<td>( p_{\text{am}} )</td>
</tr>
<tr>
<td>Hydrazine</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Acetylhydrazine</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Benzoxyldihydrazine</td>
<td>3.0</td>
<td>2.3</td>
</tr>
<tr>
<td>Isonicotinhydrazine</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>(2-Methylisonicotinyl)hydrazine</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>1-Methyl-1-isonicotinhydrazine</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Isonippecotinhydrazine</td>
<td>&lt;3</td>
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</tr>
</tbody>
</table>

**Table II**

Effect of alkyl and \( \alpha \)-methyl derivatives of isonicotinic acid hydrazide on monoamine oxidase

The listed figures indicate \( p_{\text{ox}} \) values. Assays for MO activity were performed under standard conditions; reaction vessels each contained mitochondria equivalent to 0.2 gm. of fresh liver tissue, 0.01 M tyramine, and the desired concentration of inhibitor in a total volume of 2.0 ml. with \( \frac{15}{15} \) phosphate buffer, pH 7.2. The enzymic system was incubated at 38° in an atmosphere of oxygen for 2 hours. Beef liver mitochondria control \( Q_{\text{ox}} = 128 \pm 23 \); \( Q_{\text{am}} = 39 \pm 3 \); \( O/N = 1.3 \). Mouse liver mitochondria control \( Q_{\text{ox}} = 95 \pm 12 \); \( Q_{\text{am}} = 30 \pm 3 \); \( O/N = 1.8 \).

<table>
<thead>
<tr>
<th>IAH derivative</th>
<th>Straight chain series</th>
<th>( \alpha )-Methyl series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef liver mitochondria</td>
<td>Mouse liver mitochondria</td>
</tr>
<tr>
<td></td>
<td>( p_{\text{ox}} )</td>
<td>( p_{\text{am}} )</td>
</tr>
<tr>
<td>C1</td>
<td>4.3</td>
<td>4.2</td>
</tr>
<tr>
<td>C2</td>
<td>5.9</td>
<td>5.0</td>
</tr>
<tr>
<td>C3</td>
<td>5.3</td>
<td>5.1</td>
</tr>
<tr>
<td>C4</td>
<td>5.7</td>
<td>5.6</td>
</tr>
<tr>
<td>C6</td>
<td>4.9</td>
<td>4.8</td>
</tr>
<tr>
<td>C7</td>
<td>5.0</td>
<td>4.9</td>
</tr>
</tbody>
</table>

* Iproniazid, Marsilid.

**Table III**

Effect of picolinic acid hydrazide derivatives on monoamine oxidase

Reaction vessels contained mitochondria equivalent to 0.1 gm. of fresh beef liver. \( Q_{\text{ox}} = 210 \); \( Q_{\text{am}} = 125 \); \( O/N = 1.3 \). Incubation period was 1 hour. See also legend to Table II.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( p_{\text{ox}} )</th>
<th>( p_{\text{am}} )</th>
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<tbody>
<tr>
<td>Picolinic acid hydrazide</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
<tr>
<td>1-Picolinyl-2-methylhydrazine</td>
<td>5.5</td>
<td>5.0</td>
</tr>
<tr>
<td>1-Picolinyl-2-ethylhydrazine</td>
<td>5.3</td>
<td>5.0</td>
</tr>
<tr>
<td>1-Picolinyl-2-isopropylhydrazine</td>
<td>5.3</td>
<td>4.9</td>
</tr>
</tbody>
</table>

\( N\equiv\text{CONHNCH(\text{CH}_{2})_{2}} \)

The isopropyl group, however, can be replaced by other hydrophobic residues.

From this structural picture some simple conclusions regarding the interaction between enzyme and inhibitor can be drawn. If hydrogen bonding would substantially contribute to the binding force, acylation should increase the MO-blocking power of hydrazines, because the nitrogen atom adjacent to the carbonyl residues is well suited to enter into hydrogen bonding (9). Non-

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4 Preliminary note, reference (8).
alkylated mono- and diacylhydrazides, however, do not inhibit MO.

The alkyl residues affect the MO-inhibiting activities of acylhydrazines in two ways. 
(a) They strengthen the nucleophilic nature of the nitrogen to which they are attached. This influence is expressed in the above formula by the pair of electrons to be shared with an electrophilic group of the enzyme. (b) By virtue of van der Waals' forces, they add to the binding power between enzyme and inhibitor. This is an illustration of a general principle governing the formation of complexes between enzyme and substrate or inhibitor molecules (Fig. 3 in reference (10)).

As to the nature of the electrphilic residue of the enzyme, some information may be obtained from the observation that the structure of the aliphatic group exerts the same influence on three different systems: (a) formation of transannular bonds between a ketone group and substituted ring nitrogen (11); (b) binding between 1,2-dialkylhydrazines and carbonyl compounds (1); and (c) inhibition of MO by 1,2-dialkylhydrazines (1). Tentatively, the presence of a carbonyl residue in MO as the electrophilic counterpart of the nucleophilic hydrazine nitrogen may be assumed. Since, in contrast to diamine oxidase, no readily accessible aldehyde or ketone group is found in MO, the carbonyl residue could be a part of an amide, peptide, or ester linkage.

Previously it was shown that iproniazid and substrates of MO interact with the same site of the enzyme (3, 5). Furthermore, on the basis of extensive studies of the substrate pattern of this enzyme, it was concluded that the amino group of the substrate reacts with an electrophilic residue, presumably an acyl belonging to an aromatic amino acid. Thus, it does not seem unlikely that the nucleophilic nitrogen of hydrazine interacts with this particular peptide linkage forming a part of the active site of MO. Naturally, further studies on enzymic and nonenzymic systems are required to add more light to this question.

**SUMMARY**

1. The mode of action of iproniazid on mitochondrial monoamine oxidase of mammalian liver has been investigated through a systematic analysis of the structural configuration of various series of hydrazine derivatives essential to effect inhibition.

2. Monosubstitution of the second nitrogen atom of isonicotinic acid hydrazide with alkyl groups produces optimal inhibition with the butyl derivative as evidenced by the pIso values. Methyl substitution of the alkyl group tends to diminish the inhibition.

3. The methyl, ethyl, and isopropyl derivatives of picolinic acid hydrazide are equally effective as inhibitors of beef liver mitochondrial MO.

4. None of the alkylidene and methylalkylidene isonicotinyl hydrazines, or of the 2,2-dialkyl derivatives of isonicotinic acid hydrazide or 1-(2-methylisonicotinic acid) hydrazide that were tested displayed more than a slight blocking action on MO at concentrations as high as $10^{-3}$ M. The isonicotinic acid hydrazones of pyruvic acid and glucuronic acid were likewise ineffective.

5. Monosubstitution of the second nitrogen atom of isonicotinic acid hydrazide with alkyl groups produces optimal inhibition with the butyl derivative as evidenced by the pIso values. Methyl substitution of the alkyl group tends to diminish the inhibition.

6. A concept of the functions which the various residues of the iproniazid structure serve to fulfill in the interaction with the reactive site of monoamine oxidase is discussed.

**REFERENCES**

Amine Oxidases: XVII. MODE OF ACTION OF 1-ISONICOTINYL-2-ISOPROPYLHYDRAZINE ON MONOAMINE OXIDASE
James Barsky, Winfried L. Pacha, Satyapriya Sarkar and E. Albert Zeller


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