STRUCTURE AND ACTIVITY RELATIONSHIP OF MONOAMINE OXIDASE INHIBITORS, PHENYLACETYLHYDRAZIDE DERIVATIVES

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Since the introduction of iproniazid, originally synthesized as antitubercular drug, monoamine oxidase (MAO) inhibitors have been applied to the treatment of mental depression and angina pectoris. Most of the MAO inhibitors are chemically hydrazine and hydrazide derivatives. It is suggested by many investigators that pharmacological effects of the inhibitors derive from accumulation of serotonin and catecholamines in the central nervous system and other organs. The so-called side effects of the inhibitors such as liver damage are supposed to be due to unknown direct effects. Efforts to find out less toxic inhibitors have been done, thereupon.

The present paper describes the structure-activity relationship of new MAO inhibitors, phenylacetylhydrazide derivatives by use of iproniazid and phenylisopropylhydrazine (JB-516) as control. The chemical structures of the derivatives are shown in Fig. 1.

METHODS AND MATERIALS

Male Wistar rats and male mice (DD-strain) were used. The inhibitory effect of the compounds on MAO in vitro was evaluated according to the modified method of Zeller et al. (1) by measuring the manometrical oxygen uptake of homogenates of rat brain and liver and of mitochondrial fractions of the above tissues, using tyramine, serotonin and adrenaline as substrate in which the homogenate fraction was essential to be dialyzed against phosphate buffer (1/15 M, pH 7.4) overnight before use. Reduced glutathion was added to the homogenates to prevent autoxidation of adrenaline, as was described by Blaschko et al. (2). For the determination of the inhibitory ED₅₀ on MAO, the concentration of the compounds in the reaction mixture was modified from 10⁻³ to
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10^-4 Mole. The inhibitory activity of the compounds on MAO in vivo was determined following the modified of Zeller et al. (3) as above mentioned, in which all the compounds tested were administered orally. The content of noradrenaline in rat brain was determined by use of the method described by Shore and Olin (4). The forced motor activity of mice was assayed by counting the number of falling animal in rotating cylinder devised by Toekes (5), in which the speed of rotation was changed to 2 rpm. The change of the spontaneous motor activity of mice induced by the compounds was also assayed by using the photocell cage. The oral doses of P-5307, iproniazid and JB-516 in mice were 2, 5, 10, 25, 50, 100 and 200 mg/kg. The spontaneous motor activity of six mice for each dose was observed for three hours and finally at fifteen hour after the administration. The effects of the oral pretreatment of the mice with the inhibitors on methylhexabital sodium-sleep were also studied. Nonanesthetic (40 mg/kg i.p.) and anesthetic dose (80 mg/kg i.p.) of methylhexabital sodium were administered to mice which had been pretreated with various doses of the inhibitors by two, five and fifteen hours previously. By use of modified method of Toman et al. (6), anti-electroshock activity of the compounds was studied in mice. The stimulus to produce the electroshock in mice was 25 mA in intensity and 0.2 msec in duration. The preventive activity of the compounds against metrazol-shock in mice was studied using the modified method of Swinyard et al. (7). The intraperitoneal dose of metrazol required to produced 98% convulsion in mice was 175 mg/kg. The depression of the tonic extensor reflex of the hind limb was used as a measure of the activity of the compounds. The subacute toxicity of the compounds was studied in rats which have received daily oral doses of 5, 10 and 30 mg/kg of P-5307 and iproniazid, and 2, 5 and 10 mg/kg of JB-516 for five weeks. Five rats were used for each of the doses. The increase of the body weight during the period was compared with that of control animals. After the termination of the experiment the animals were sacrificed and the tissues of lungs, liver, kidney, adrenals, spleen, heart, stomach, small intestine and testis were macro- and microscopically examined. Acute toxicity of the compounds was studied in rats and mice. The L.D_{50} of the compounds was calculated following the method of Litchfield and Wilcoxon (8). All the compounds tested in the experiments were synthesized by Drs. H. Takamatsu and S. Umemoto in synthetic division of this Laboratories.

RESULTS AND DISCUSSION

1. Inhibition of MAO in vitro

The inhibitory effect of fourteen derivatives of phenylacetylhydrazides on MAO in vitro are summarized in Tables 1 and 2. Substitutions of one hydrogen at the terminal amino nitrogen in the molecule all increase the inhibitory effect. Especially, the activity of the benzyl, phenethyl and isopropyl groups is markedly increased in the described order. The activity of the benzyl derivative, N-benzyl-N' phenylacetylhydrazide (P-5307) is as strong as that of JB-516 and is more active than that of iproniazid. The phenethyl and isopropyl derivatives are as active as iproniazid. The modification of the
inhibitory activity by the introduction of alkyl and phenyl groups into α-carbon in N-isopropyl-N'-phenylacetylhydrazide is shown in Table 2-1. Any such substitutions decrease the activity. The effects of introduction of the aliphatic and aromatic groups into one or two hydrogens at α-carbon in N'phenylacetylhydrazide is summarised in Table 2-2. The results are also disappointing, the decrease in the activity. Even the monosubstitution with ethyl or phenyl group decreased the activity.

From the results it is concluded that any substitution of the hydrogen at α-carbon of phenylacetylhydrazide decreases the MAO inhibitory activity irrespective of the substitution of the hydrogen at the terminal amino nitrogen. While, introduction of any group into one hydrogen at the terminal amino nitrogen increases the activity.

Further, P-5307, iproniazid and JB-516 are incubated with whole homogenates and mitochondrial fractions prepared from rat brain and liver using adrenaline and serotonin as substrate. As is shown in Table 3, a marked difference of the MAO inhibition is observed between the homogenates and the mitochondrial fractions. The mitochondrial enzyme is suppressed by P-5307 and iproniazid more than that of the homogenates. The similar result was obtained on iproniazid by Zeller et al. (1). The inhibitory effect of JB-516 on both MAO preparations is almost same. The liver homogenate is more sensitive to P-6307 and iproniazid than the brain homogenate, while JB-516 has no marked difference in the sensitivity though the latter compound is rather more active to brain homogenate.
2. **Inhibition of MAO in vivo**

One hundred milligram per kilogram of P-5307 and iproniazid, and 30 mg/kg of JB-516 are administered intraperitoneally to five rats, respectively. Thereafter, animals are killed 1 and 3 hours, and 2, 5 and 10 days after the administration and the MAO activity of the brain and liver is examined. As is shown in Fig. 2, the compounds show a long lasting inhibition of MAO. The time course of the inhibition shows no marked difference between them. The duration of brain MAO inhibition is found to be longer than that of liver MAO inhibition.

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**Table 3. Inhibition of monoamine oxidase in vitro.**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Source</th>
<th>Enzyme</th>
<th>ED$_{50}$</th>
<th>Serotonin</th>
<th>Adrenaline</th>
<th>Tyramine</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>molar concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-5307</td>
<td>Brain</td>
<td>Homogenate</td>
<td>1.1 × 10$^{-3}$</td>
<td>9.9 × 10$^{-3}$</td>
<td>5.1 × 10$^{-5}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondria</td>
<td>4.8 × 10$^{-4}$</td>
<td>5.5 × 10$^{-3}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Homogenate</td>
<td>8.1 × 10$^{-4}$</td>
<td>3.2 × 10$^{-3}$</td>
<td>7.6 × 10$^{-5}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondria</td>
<td>5.8 × 10$^{-4}$</td>
<td>9.8 × 10$^{-5}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iproniazid</td>
<td>Brain</td>
<td>Homogenate</td>
<td>2.2 × 10$^{-4}$</td>
<td>%×10$^{-5}$</td>
<td>9.2 × 10$^{-4}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondria</td>
<td>9.2 × 10$^{-4}$</td>
<td>1.1 × 10$^{-3}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Homogenate</td>
<td>1.5 × 10$^{-4}$</td>
<td>6.3 × 10$^{-3}$</td>
<td>6.6 × 10$^{-5}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondria</td>
<td>9.8 × 10$^{-4}$</td>
<td>4.7 × 10$^{-3}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JB-516</td>
<td>Brain</td>
<td>Homogenate</td>
<td>2.5 × 10$^{-4}$</td>
<td>3.3 × 10$^{-3}$</td>
<td>8.5 × 10$^{-5}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondria</td>
<td>1.0 × 10$^{-3}$</td>
<td>2.6 × 10$^{-4}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Homogenate</td>
<td>3.5 × 10$^{-4}$</td>
<td>8.6 × 10$^{-4}$</td>
<td>7.1 × 10$^{-4}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mitochondria</td>
<td>1.1 × 10$^{-4}$</td>
<td>2.5 × 10$^{-4}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ED$_{50}$ means molar concentration producing 50 percent inhibition of monoamine oxidase. Methods: see text.
3. Effects of the MAO inhibitors on the content of brain noradrenaline

The content of noradrenaline in the brain of five rats which received daily dose of 30 mg/kg of P-5307 and iproniazid, and 5 mg/kg of JB-516 for five weeks is determined. The level of the brain noradrenaline is all elevated, as is shown in Table 4. The rats which have received the last dose of the iproniazid only show a marked piloerection and other signs of restlessness. However, the animals show the least level of noradrenaline increase in the brain. From the results it is suggested that the level of noradrenaline produced by the administration of the compounds is not the determining factor for the central excitement. Brodie et al. (9) reported, however, that excitement by MAO inhibitors might not be concerned with serotonin but noradrenaline.

4. Other central effects of the compounds

1) Effects on the spontaneous and forced motor activity in mice

The effects of the compounds on the spontaneous and forced motor activity in mice were studied by use of the photocell cage and rotating cylinder. The motor response of the animal in photocell cage to P-5307 and iproniazid does not significantly differ from that of the untreated animal, whereas the same response to JB-516 is a marked increase in the spontaneous movement which develops immediately after the administration. The response to JB-516 is mimiced closely but is about one-fifth of the same

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**Table 4. Effect of daily oral administration of MAO inhibitors on noradrenaline content in rat's brains.**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose mg/kg/day</th>
<th>No. of rats</th>
<th>Noradrenaline ± S.E. μg/g brain tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>5</td>
<td>0.531 ± 0.0305</td>
</tr>
<tr>
<td>P-5307</td>
<td>30</td>
<td>5</td>
<td>0.638 ± 0.0428</td>
</tr>
<tr>
<td>Iproniazid</td>
<td>30</td>
<td>5</td>
<td>0.625 ± 0.0352</td>
</tr>
<tr>
<td>JB-516</td>
<td>5</td>
<td>5</td>
<td>0.738 ± 0.0321</td>
</tr>
</tbody>
</table>

Noradrenaline content was determined at the end of 5 weeks during which the drugs were daily and orally administered.

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**Fig. 3. Effects on mice performance in photocell cage.**
response to methamphetamine. The results obtained are summarized in Fig. 3. The similar results were reported recently by Furgiule et al. (10). However, they described that the intraperitoneal administration of iproniazid to mice produced depression of the spontaneous movements. Then, using rotating cylinder, effects of the compounds on the forced motor activity in mice are examined. The animal responses in rotating cylinder to P-5307, iproniazid and JB-516 show no significant difference from that of the saline-treated animal.

2) Effects of the compounds on barbiturate sleep in mice

Various doses of the compounds are administered orally to mice 2, 5 and 15 hours before the intraperitoneal injection of 40 mg/kg of methylhexabital sodium, by which itself the anesthetic effect is not produced. However, these pretreatments of the animal with compounds do not evoke any anesthetic effect. On the other hand, as is shown in Fig. 4, higher doses (100 and 200 mg/kg) of iproniazid and JB-516 prolong the barbiturate sleep produced by 80 mg/kg of methylhexabital sodium. The pretreatment of the animal with higher dose of 200 mg/kg of P-5307 causes no prolongation of the barbiturate sleep. The lack of the prolongation of P-5307 to barbiturate sleep may derive from the inert action of the compound on the enzymes of liver which degrade barbiturate metabolically.

3) Anticonvulsant effect of the compounds

The anticonvulsant activities of the compounds are evaluated in mice by the prevention of the tonic extensor reflexes of the hind limb produced by electroshock or metrazol convulsion. The tonic extensor reflex produced by the electroshock is not prevented by any doses of the compounds, while the same reflex produced by metrazol injection is slightly depressed by P-5307 and JB-516. However, the therapeutic indices of both compounds are markedly smaller than that of the antiepileptics. These results
are summarized in Table 5. The conclusion of Prockop et al. (11) that MAO inhibitors show an anticonvulsant effect and the effect is related to the elevated brain noradrenaline and serotonin, may require further studies.

5. Toxicity studies

1) Acute toxicity

The general toxicity of the MAO inhibitors has been reported to relate with its inhibitory activity on MAO (12). Though the MAO inhibitory activity of P-5307 proves to be strongly active in vitro as well as in vivo, the acute toxicity of the compound in rats and mice is comparatively less, compared with those of iproniazid and JB-516, as is shown in Table 6. There is no clear relation between MAO inhibitory activity and acute toxicity in experimental animals.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Oral ED₅₀ mg/kg</th>
<th>LD₅₀/ED₁₀</th>
<th>Oral ED₅₀ mg/kg</th>
<th>LD₅₀/ED₁₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-5307</td>
<td>&gt;400</td>
<td>&lt;1.92</td>
<td>385 (313-455)</td>
<td>1.99</td>
</tr>
<tr>
<td>Iproniazid</td>
<td>&gt;400</td>
<td>&lt;1.85</td>
<td>&gt;400</td>
<td>&lt;1.85</td>
</tr>
<tr>
<td>JB-516</td>
<td>&gt;200</td>
<td>&lt;0.75</td>
<td>125 (98-153)</td>
<td>1.20</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>30 (31-46)</td>
<td>11.5</td>
<td>30 (22-37)</td>
<td>15.0</td>
</tr>
<tr>
<td>Phenuronic</td>
<td>75 (62-84)</td>
<td>65.0</td>
<td>65 (51-78)</td>
<td>77.0</td>
</tr>
<tr>
<td>Dilantin</td>
<td>16 (12-19)</td>
<td>38.1</td>
<td>&gt;750</td>
<td>&lt;0.812</td>
</tr>
<tr>
<td>Tridione</td>
<td>&gt;1,000</td>
<td>&lt;3.72</td>
<td>338 (305-372)</td>
<td>11.0</td>
</tr>
</tbody>
</table>

2) Subacute toxicity

The daily oral doses of 5, 10 and 30 mg/kg of P-5307 and iproniazid, and 1, 2 and 5 mg/kg of JB-516 are given to the male rats, consisted of five rats for each dose, for 5 weeks. During this period, the increase of the body weight of the rats was examined. Thirty milligram per kilogram of iproniazid decreases distinctly the growth rate. P-5307 and JB-516 do not affect the body weight gain except that 30 mg/kg of P-5307 produced more increase in the body weight than control animal. At fourth week of the administration the bromosulfalcin retention test is carried out in the rats which have
received highest doses of the compounds and BSP values obtained were as follows: 2% for control animal, 1% for P-5307, 6% for iproniazid and 4.8% for JB-516. All these values are within the normal range. At the end of the fifth week of the administration the animals are sacrificed and the extirpated tissues such as heart, lungs, liver, kidney, stomach, small intestine and testis are examined macro- and microscopically. The only organ that proves pathological is the liver. Though the slight infiltration of the small cell in the Glisson's sheath produced by iproniazid and slight cloudy swelling produced by JB-516 are observed, any pathological signs are not detected in the liver of rat which receives P-5307. No demonstrable effects are obtained on the other tissues after the administration of P-5307.

**SUMMARY**

The inhibitory effect of total fourteen derivatives of phenylacetylhydrazide on MAO were examined using rat brain and liver enzyme preparations in vitro and in vivo. N-benzyl-N'-phenylacetylhydrazide (P-5307) was found to be most active MAO inhibitor among derivatives assayed. MAO-inhibition and CNS effects of P-5307 were comparatively investigated using iproniazid and JB-515 as control.

1. The introduction of any aliphatic and aromatic groups into one hydrogen at the terminal amino nitrogen of phenylacetylhydrazide molecule increased the inhibitory activity. Substitutions of one or two hydrogen at α-position of the molecule with aliphatic or aromatic group decreased the activity.

2. N-phenethyl-N’-phenylacetylhydrazide (P-5305) and N-isopropyl-N’-phenylacetylhydrazide (P-5613) were as active as iproniazid, while P-5307 was more active than iproniazid.

3. P-5307 and iproniazid suppressed MAO activity of liver and brain mitochondria.
more than that of both original homogenates, whereas, JB-516 inhibited the MAO of the homogenates and mitochondria of brain and liver in the same degree.

4. P-5307 and iproniazid were more active to liver MAO than to brain MAO. No such difference in the sensitivity was observed in JB-516.

5. The duration of inhibitory effect of P-5307, iproniazid and JB-516 on brain MAO in vivo was longer than that on liver MAO.

6. Subacute administration of P-5307, iproniazid and JB-516 to rats increased the brain noradrenaline. Rats which received iproniazid showed a piloerection and motor excitement, though the increase in cerebral noradrenaline was the lowest.

7. No appreciable effects of P-5307, iproniazid and JB-516 were observed on the mice performance of forced motor activity using rotating cylinder. The spontaneous motor activity of mice in photocell cage was not affected by P-5307 and iproniazid, but was markedly increased by JB-516.

8. The pretreatment of P-5307, iproniazid and JB-516 did not induced sleep in mice received nonanesthetic dose of methylhexabital. However, the sleep induced by anesthetic dose of the barbiturate was prolonged by iproniazid and JB-516 but not by P-5307.

9. The tonic extensor reflexes in mice produced by electroshock or metrazol were not significantly suppressed by P-5307, iproniazid and JB-516.

10. The acute toxicity studies excluded the correlation between the toxicity and inhibitory effect on MAO. P-5307 proved the lowest toxicity in rats and mice.

11. The subacute toxicity studies excluded damaging effect of P-5307 on the tissues such as liver, kidney and testis in rats. While, iproniazid and JB-516 caused a slight cloudy swelling and cell infiltration in the Glisson's sheath in liver.

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