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COMPARATIVE EFFECTS OF MONOAMINE OXIDASE INHIBITORS ON MONOAMINE OXIDASE AND DIAMINE OXIDASE*

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Abstract—The effect of several monoamine oxidase (MAO) inhibitors and related compounds on guinea pig liver MAO and diamine oxidase (DAO) was examined *in vitro* and after administration to animals. The results showed that the MAO inhibitors possessing the hydrazine moiety inhibited both MAO and DAO, while two phenyl*cyclo*propylamine MAO inhibitors did not affect DAO. Hydrazine derivatives which did not block MAO retained a DAO inhibitory action. After administration of iproniazid to rats, DAO was inhibited long after the reported time for the drug to disappear from tissues; this finding suggests that the action of iproniazid on DAO, as well as on MAO, is "irreversible".

THE question of the mechanisms involved in the diverse clinical effects induced by monoamine oxidase (MAO) inhibitors is an intriguing one. Included among the clinical effects are orthostatic hypotension, abatement of anginal pain and an antidepressant effect.[1] In laboratory animals a degree of central stimulation, anticonvulsant and sympathomimetic effects have been observed.[1]

The diversity of the pharmacologic effects emphasizes the desirability of a more complete knowledge of the actions, other than inhibition of MAO, which might be induced by these drugs.

In the present studies we have investigated the effects on diamine oxidase (DAO) *in vivo* and *in vitro* of a variety of drugs (Fig. 1) including: (a) MAO inhibitors containing the hydrazine moiety, (b) non-hydrazine MAO inhibitors, and (c) hydrazine derivatives which are not MAO inhibitors.† The experiments demonstrate that the hydrazine moiety confers DAO blocking activity, whether or not the molecule also blocks MAO activity, while the non-hydrazine MAO inhibitors tested do not block DAO. The studies further suggest that the action *in vivo* of iproniazid on DAO as well as MAO is "irreversible".

METHODS

Histamine was estimated fluorometrically by the method of Shore *et al.*[2] and serotonin by the method of Bogdanski *et al.*[3]

DAO activity was estimated by measurement of the rate of disappearance of histamine added to tissue homogenates, while MAO activity was measured in a similar fashion by following the disappearance of serotonin.

* A preliminary report of these findings was presented before a meeting of the American Society for Pharmacology and Experimental Therapeutics, Chicago, April, 1960.

† Iproniazid, isoniazid, Ro 4-1634 and Ro 4-1038 were kindly supplied by Hoffmann-La Roche, Inc., and SKF 385 and SKF 556 by Smith, Kline and French Laboratories.

Tissues were homogenized in 9 vols. of 0·2 M phosphate buffer, pH 7·2. After pre-incubation in a Dubnoff metabolic shaker for 15 min at 37 °C in an atmosphere of air, histamine or serotonin was added to make a final concentration of 7·5 μg/ml histamine or 12·5 μg/ml serotonin. Samples were removed 20 min later for serotonin assay or 60 min later for histamine assay.

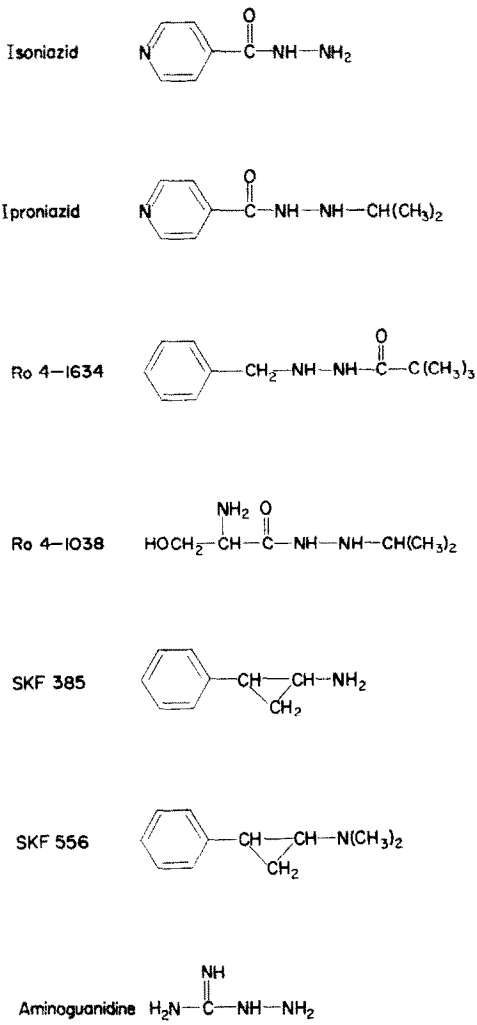

FIG. 1. Monoamine oxidase inhibitors

Inhibition of the enzymes *in vitro* was carried out by addition of inhibitors to the homogenates at the beginning of the 15-min pre-incubation period. Homogenates to which no inhibitor was added served as controls. The extent of inhibition *in vivo* after intraperitoneal administration of the inhibitors was measured in a similar manner; tissue homogenates from untreated animals served as controls.

RESULTS

Inhibition of DAO and MAO in vitro

Guinea pig liver was used in these experiments since it is a tissue rich in both MAO and DAO. The extent of inhibition of DAO and MAO activities by various concentrations of the drugs is shown in Table 1. A marked inhibition of DAO was obtained

TABLE 1. INHIBITION OF GUINEA PIG LIVER MAO AND DAO *in vitro*

Inhibitor	Molar Concentration of Inhibitor							
	10^{-3}		10^{-4}		10^{-5}		10^{-6}	
	MAO	DAO	MAO	DAO	MAO	DAO	MAO	DAO
Iproniazid	98	75	11	68	0	16	0	10
Ro 4-1634	100	100	100	100	100	100	0	27
Ro 4-1038	100	100	78	86	3	39	0	9
SKF 385	100	13	100	0	95	0	0	0
SKF 556	88	15	87	4	79	0	1	0
Aminoguanidine	0	100	0	100	0	100	0	100
Isoniazid	2	93	2	60	0	14	0	6

Experiments were performed as described under "Methods". Each value represents the average percentage inhibition in from three to six experiments.

with all of the hydrazine derivatives, but not with the phenyl*cyclo*propylamines. Conversely, a marked inhibition of MAO was obtained with the phenyl*cyclo*propylamines and certain of the hydrazine derivatives. Aminoguanidine and isoniazid did not inhibit MAO even at high concentrations.

Inhibition of DAO and MAO in vivo

Measurement of DAO and MAO activity of liver from guinea pigs treated with the various drugs revealed a picture of inhibition (Table 2) very similar to that seen in the studies *in vitro*. All of the hydrazine derivatives caused a marked inhibition of DAO.

TABLE 2. INHIBITION OF GUINEA PIG LIVER MAO AND DAO *in vivo*

Inhibitor	1 hr		24 hr	
	MAO	DAO	MAO	DAO
Iproniazid (100 mg/kg)	100	100	100	94
Ro 4-1634 (100 mg/kg)	100	100	100	46
Ro 4-1038 (100 mg/kg)	100	100	100	71
SKF 385 (3 mg/kg)	100	0	6	0
SKF 556 (20 mg/kg)	62	0	14	0
Aminoguanidine (20 mg/kg)	0	91	0	78
Isoniazid (100 mg/kg)	0	70	0	80

Experiments were performed as described under "Methods". Each value represents the average percentage inhibition in from three to six experiments.

but only certain of these compounds caused an inhibition of MAO. The phenycyclopropylamines were highly active in blocking MAO, but were devoid of DAO blocking activity.

After administration of 100 or 195 mg of iproniazid per kg, to rats, the activity of DAO in the small intestine, the major source of DAO in this species, was strongly inhibited for at least 48–72 hr (Table 3).

TABLE 3. INHIBITION OF RAT SMALL INTESTINE DAO *in vivo* BY IPRONIAZID

Time after iproniazid (hr)	Dose of iproniazid	
	100 mg/kg	195 mg/kg
18	92	—
24	77	85
48	59	67
72	57	—

Experiments were performed as described under "Methods". Each value represents the average percentage inhibition in from three to six experiments.

DISCUSSION

It is possible to draw certain conclusions from the experiments with the representative compounds described in this paper.

Hess *et al.*[4], showed that 24 hr after administration of iproniazid, 195 mg/kg, to rats, the drug had essentially disappeared from the tissues, but that inhibition of MAO persisted much longer. These results constituted one of the means of demonstration of the "irreversible" nature of iproniazid-induced inhibition of MAO. The present results demonstrate that iproniazid, which has been reported previously to block DAO *in vitro*,[5] inhibited DAO *in vivo* for at least 48 hr after the same dose used by Hess *et al.*[4] After only one-half this dose (100 mg/kg), inhibition of DAO activity again persisted for at least 48–72 hr. The results are suggestive that DAO as well as MAO is "irreversibly" inhibited by iproniazid.

The data also demonstrate that not only iproniazid, but also other MAO inhibitors which contain the hydrazine moiety, can block DAO as well as MAO, *in vitro* and *in vivo*. Indeed, DAO was blocked *in vitro* more strongly than MAO by certain concentrations of the MAO inhibitors, iproniazid, Ro 4-1038, and Ro 4-1634. It is noteworthy that the hydrazine group need not be in the free or "carbonyl reagent" form for blockade of DAO, as might be suspected from reports of inhibition of DAO by carbonyl reagents such as semicarbazide.[6] The present results suggest that inhibition of DAO by hydrazine containing molecules does not necessarily proceed by reaction with a carbonyl group.

The absence of DAO inhibition at high concentrations of the potent MAO inhibitors SKF 385 and SKF 556 demonstrates that DAO inhibition is not necessarily a corollary of MAO inhibition.

A further separation of enzyme inhibitory activity is obtained with isoniazid and with aminoguanidine, compounds which long have been known to inhibit DAO.[5, 7]

The inability of guinea pig liver to metabolize histamine *in vitro* in the presence of aminoguanidine or isoniazid (Table 1), and the unaltered ability to destroy histamine

during complete blockade of MAO activity by the addition of SKF 385 or SKF 556 (Table 1), suggest that guinea pig liver MAO is incapable of metabolizing histamine. Similar results were obtained when a homogenate of beef liver was substituted for guinea pig liver. The results differ from those of others who used very high substrate concentrations (10^{-2} M) and on the basis of manometric measurements reported that beef liver MAO could metabolize histamine.[8]

It is of interest that although some of the hydrazine-containing MAO inhibitors (Ro 4-1634 and Ro 4-1038) inhibit guinea pig liver DAO more strongly than MAO at low concentrations *in vitro*, the action of these drugs *in vivo* appears to be more rapidly reversed in the case of DAO than MAO, suggesting either a different mode of action of the inhibitors or a difference in the rates of enzyme synthesis.

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