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INHIBITION OF DIAMINE OXIDASE *IN VIVO* BY HYDRAZINE DERIVATIVES

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Abstract—The *in vivo* activity of diamine oxidase (DAO) in rat intestine was measured manometrically after intraperitoneal administration of several hydrazine derivatives. The following results were obtained:

1. Iproniazid (72 μM/kg) caused strong inhibition of the enzyme, normal activity being restituted after about 48 hr. Monoamine oxidase (MAO) in brain, however, was inhibited for more than 7 days under the same conditions.
2. DAO inhibition induced by iproniazid could be reversed only partially and after prolonged dialysis.
3. Iproniazid inhibited DAO nearly ten times more strongly than isoniazid, almost equally to semicarbazide but much less than aminoguanidine.
4. There exists no parallelism between the *in vivo* and the *in vitro* potency of the DAO inhibitors investigated.

SEVERAL hydrazine derivatives, e.g. N^2-*iso*propyl*iso*nicotinic acid hydrazide (iproniazid, Marsilid*, are potent inhibitors of monoamine oxidase (MAO) *in vitro*[1–4] and *in vivo*.[4–10] Furthermore these compounds are inhibitors of diamine oxidase (DAO) *in vitro*[1, 11–13] and some of them also *in vivo* (e.g. aminoguanidine, *iso*nicotinic acid hydrazide (isoniazid, Rimifon*)).[14] Iproniazid possibly causes DAO inhibition *in vivo*, too. Evidence for this effect is, however, only indirect.[15–19] Besides, nearly all the results have been obtained by using histamine, the bulk of which is probably not metabolized through DAO in all tissues.[20] Other authors could not find DAO inhibition by iproniazid *in vivo*.[21]

It is conceivable that some of the pharmacological and clinical effects of iproniazid and related compounds in the central nervous system are caused by MAO inhibition.[4,22] Other actions of these drugs (e.g. hypotension, effect on gastric secretion) are, however, difficult to explain by this mechanism and might possibly be due in part to DAO inhibition. In order to elucidate further the mechanism of the clinical and pharmacological action of hydrazine derivatives more information on the *in vivo* activity of these compounds is therefore desirable. In the present paper the effect of iproniazid and some other hydrazine derivatives was studied *in vivo* on intestinal DAO and compared to their action *in vitro*. Thereby cadaverine served as substrate. Furthermore the action of iproniazid on DAO of the small intestine was compared to that on brain MAO.

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* Trade name.

METHODS

Experiments in vivo

1. *DAO activity.* After 24 hr fasting, female Wistar rats weighing from 60 to 80 g received a single i.p. injection of the different compounds* in aqueous solution. The inhibitors were applied in three doses, each dose was given to six animals 4 hr prior to decapitation (if not otherwise stated). Untreated rats served as controls. The whole intestines were freed from the contents by perfusion with 25 ml sodium potassium phosphate buffer (Merck p.a. 0·067 M, pH 7·3), softly pressed out and blotted between filter paper. The pooled intestines of each group were cut into small pieces and homogenized in one volume (1 g organ + 1 ml. buffer) of ice-cold buffer by a Servall Omni-mixer (16,000 rev/min for 45 sec). The homogenate was centrifuged at 29,000 × *g* (Servall SS-1) in a cold room for 30 min, the supernatant decanted and kept overnight at + 4 °C after addition of one drop of octyl alcohol (in order to prevent putrefaction and to inhibit MAO).[23]

The activity of the enzyme was measured in Warburg manometers (37·5 °C; O_2 atmosphere, shaking frequency 120/min) during 90 min after addition of the substrate (cadaverine hydrochloride (Roche), final concentration 0·01 M). Each vessel contained enzyme quantities from about 0·8 g fresh tissue, the centre well 10% KOH with filter paper. The activity was expressed as Q_{O_2}, the initial velocity of oxygen uptake calculated as microlitres per hour per gramme wet tissue and corrected for a blank oxygen uptake. Calculation of the ED_{50} (dose for 50 per cent reduction of Q_{O_2}) by regression analysis[24, 25] from at least three series of experiments. The fiducial limits[26] are given for 95 per cent probability.†

2. *MAO activity.* Pretreatment of rats as described under (1). The brain and the medulla oblongata of eight animals, for each dose of the drug, were washed in ice-cold 0·25 M sucrose (Baker p.a.), blotted on filter paper, pooled and homogenized in 4 vols. of 0·25 M sucrose with a Potter–Elvehjem homogenizer (rotating part in Teflon, clearance 0·004–0·006 in. (Kontes Glass Co., Vineland, N.J.)). After a first slow centrifugation (30 min at about 500 × *g*) the supernatant was again centrifuged (30 min at 30,000 × *g*) and the particulate fraction including the fluffy layer resuspended in buffer by stirring with a glass rod. After further centrifugation (20 min at 12,000 × *g*) the "washed" sediment was strongly shaken in buffer until the suspension appeared perfectly homogeneous. All operations were performed at from + 2 ° to 4 °C.

The MAO activity was measured in Warburg manometers as described under (1). The flasks contained particulate matter corresponding to about 0·8 g fresh brain and tyramine hydrochloride (Roche) as substrate (0·0091 M final concentration). The results were expressed as microlitres of O_2 per hour per gramme of original wet tissue, corrected for a blank oxygen uptake. The reaction was linear for at least 60 min.

Experiments in vitro

The enzyme sources were fresh recentrifuged solutions in buffer of DAO acetone

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* Iproniazid phosphate and isoniazid, F. Hoffmann-La Roche & Co. Ltd., Basel; semicarbazide hydrochloride, Merck & Co.; aminoguanidine hydrogen carbonate, Fluka A.G., St. Gallen. N^1 : N^2-di*iso*propylhydrazine hydrochloride, *iso*propylhydrazine hydrochloride and N^1-*iso*nicotinyl-N^1 : N^2 di*iso*propylhydrazine as well as N^1 : N^2-di*iso*propylsemicarbazide were synthesized in the Chemical Research Departments of F. Hoffman-La Roche & Co. Ltd., Basel and Nutley, by Drs. O. Straub, P. Zeller and H. H. Fox.

† Calculation and statistical analysis carried out by Mr. G. Güetli.

powder[27] from hog kidney cortex as well as DAO from rat intestine as described above. The activity of DAO was measured by manometry as described above; the inhibitors were added to the enzyme solution 15 min before addition of the substrate.[28, 29] The efficiency of an inhibitor was expressed by the molar concentration that produced 50 per cent reduction of Q_{O_2} as well as by p_I50 (negative logarithm of that inhibitor concentration). These values were established graphically after measuring the effect of different inhibitor concentrations.

RESULTS

1. *Inhibition of DAO and MAO* in vivo *by iproniazid* (*Fig.* 1)

A single i.p. dose of 72 μM/kg (20 mg/kg) iproniazid phosphate produced a strong inhibition of DAO with a maximal effect between $1\frac{1}{2}$ to 16 hr after administration of the drug. About 50 per cent inhibition was present already after 15 min, complete recovery of the enzyme activity after about 48 hr. Inhibition of brain MAO in the same animals developed only after 4 hr and was maximal up to 16 hr. Contrary to the

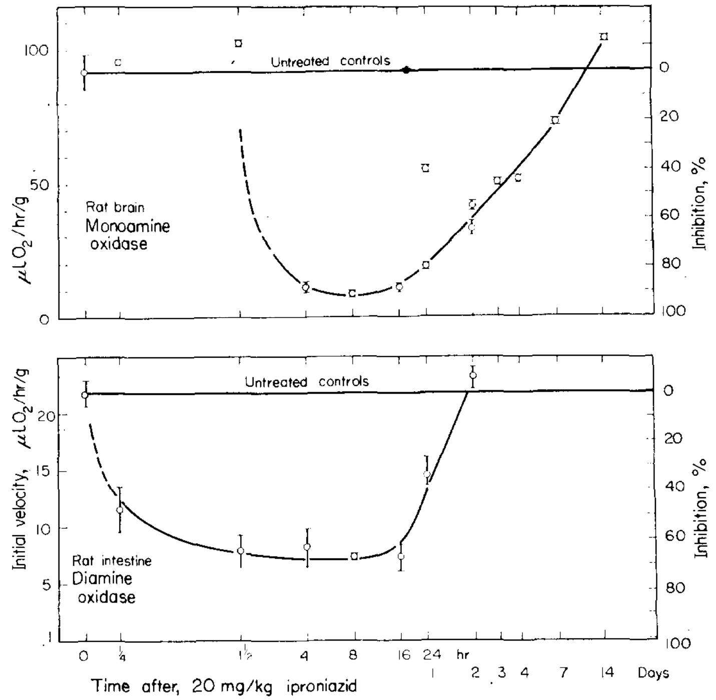


FIG. 1. Iproniazid induced inhibition of MAO and DAO *in vivo* in rats as function of time

Abscissa: time in hours or days in a logarithmic system. Ordinate: left, absolute activity μlO_2 uptake hr^{-1} g^{-1}; right, percentage inhibition. Vertical lines: standard errors of mean values. At zero time 72 μM/kg iproniazid was injected i.p. Each value is derived from a pool of eight animals; the average value of untreated rats killed at the same time intervals served as control. MAO and DAO determinations were carried out in the same animals.

inhibition of DAO the brain MAO was depressed for more than 7 days. The maximum inhibition of brain MAO was of the same order as that of DAO.

2. *Reversibility of DAO inhibition induced by iproniazid* (*Table* 1)

Dialysis of homogenates for 14 hr resulted in a very slight decrease of the DAO activity (14 per cent). Only a very prolonged moving dialysis (5 ml enzyme solution against 5 × 2 l. buffer within 3 days) decreased the inhibition to a marked degree (42 per cent).

TABLE 1. INHIBITION OF DAO BY IPRONIAZID *in vitro* WITH AND WITHOUT DIALYSIS

Enzyme preparation		Homogenate of rat intestine	Acetone powder of hog kidney cortex	
Duration of the experiment (hr)		14	14	72
Inhibition (%)	without dialysis	89	98	91
	with dialysis	75	86	49
Reversibility of the iproniazid inhibition (%)		14	12	42

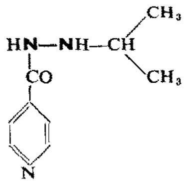
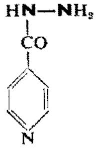
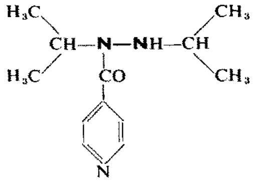
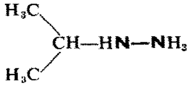
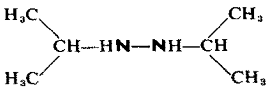
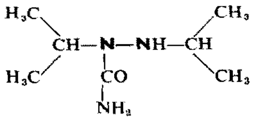
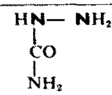
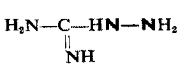
Iproniazid (final concentration 0·01 M) or distilled water (control) were added to a total volume of 5 ml enzyme solution. 10 min later dialysis was started in slowly-moving cellulose casings (Visking Co., Chicago, Ill.) for 14 hr against 2 l. buffer or for 3 days against 5 × 2 l. buffer. All operations were done at from + 2 to + 4 °C. The activity of the samples with iproniazid was compared to controls treated correspondingly.

3. *Comparison of DAO inhibitors* in vivo *and* in vitro (*Table* 2)

In vitro the order of potency of the inhibitors was the following: aminoguanidine > *iso*propylhydrazine = semicarbazide ≫ di*iso*propylhydrazine = isoniazid = iproniazid. Nicotinyldi*iso*proplhydrazine and di*iso*propylsemicarbazide were inactive. DAO from rat intestine as well as purified DAO from hog kidney were equally inhibited by semicarbazide and isoniazid. The affinity of both enzyme preparations for putrescine and cadaverine was also similar. Therefore the *in vitro* results obtained with hog kidney DAO seem to be equivalent to those with enzyme from rat intestine.

In vivo the order of potency of DAO inhibition was quite different from that found *in vitro*, namely: aminoguanidine ≫ *iso*propylhydrazine > di*iso*propylhydrazine ≥ iproniazid = semicarbazide > di*iso*propyl-semicarbazide = *iso*nicotinyldi*iso*propylhydrazine > isoniazid. Thus, di*iso*propylhydrazine and iproniazid had relatively more potency *in vivo* than *in vitro*, semicarbazide, however, less. The two compounds which showed no effect *in vitro* (*iso*nicotinyldi*iso*propylhydrazine and di*iso*propylsemicarbazide) had a measurable inhibitory action *in vivo*, even more than isoniazid which was the weakest of all the compounds investigated.

TABLE 2. COMPARISON OF DAO INHIBITORS *in vivo* AND *in vitro*

Method		*in vivo*		*in vitro**		
Inhibitor	Structure	ED_{50} (μM/kg) (fiducial limits)	ED_{50} iproniazid / ED_{50} inhibitor	pI50	Final inhibitor conc. for 50% inhibition (μM)	μM iproniazid / μM inhibitor
Iproniazid		5·5 (2·8–11·6)	1·00	4·2	0·07	1·00
Isoniazid		51·7 (19·0–91·7)	0·11	4·0	0·09	0·8
N^1-*iso*Nicotinyl-N^1: N^2-di*iso*propylhydrazine		19·4 (10·0–66·0)	0·28	<2	>10	<0·01
*iso*Propylhydrazine		1·0 (0·4–1·3)	5·5	6·1	0·00080	87·5
N^1 : N^2-Di*iso*propylhydrazine		2·4 (2·0–3·1)	2·3	4·6	0·025	2·8
N^1 : N^2-Di*iso*propylsemicarbazide		15·2 (8·8–24·5)	0·36	<2	>10	<0·01
Semicarbazide		6·5 (3·5–10·4)	0·85	6·1	0·00085	82
Aminoguanidine	 $H_2N{-}C({=}NH){-}HN{-}NH_2$	0·07 (0·027–0·12)	78·6	7·2	0·00007	1080

* The values for 50 per cent inhibition described above correspond approximately to the following figures in the literature found under various conditions: iproniazid pI50 ≤ 4·0,[1],[31] 0·005 M;[12] isoniazid pI50 = 4·0;[1],[31] N^1 : N^2-di*iso*propylhydrazine pI50 = 3·7;[30] semicarbazide 0·00004 M,[32] 0·00005 M,[33] 0·00001 M;[12] aminoguanidine 0·00000005 M,[33] 0·0000005 M.[12]

DISCUSSION

The present investigations demonstrate by direct determination of DAO activity in the rat intestine that certain hydrazine derivatives inhibit this enzyme *in vivo.*

Thus, earlier findings of DAO inhibition based on indirect measurements are confirmed.[15–19] Furthermore it is shown that there exists no parallelism between the *in vivo* and the *in vitro* potency of DAO inhibitors. Thus, the quotient:

$$\frac{\text{final concentration for 50 per cent inhibition } \textit{in vitro} \times 100}{\text{ED}_{50} \textit{ in vivo}}$$

(Table 3) is high (1 or more) for iproniazid, N^1-*iso*nicotinyl-N^1 : N^2-di*iso*propylhydrazine, N^1 : N^2-di*iso*propylhydrazine and N^1 : N^2-di*iso*propylsemicarbazide, indicating a relatively stronger inhibition *in vivo* compared to *in vitro*. On the other hand the quotient is low for isoniazid, semicarbazide and aminoguanidine (0·18 and less) suggesting a relatively weaker effect *in vivo* compared to *in vitro*. The change of the relative potency of some compounds *in vivo* might be caused by metabolic alterations or a different degree of penetration to the reactive site of the enzyme. Concerning MAO inhibition there exists some evidence that the *iso*propylhydrazine moiety is the active part of the iproniazid molecule[3, 10] and that it possibly splits off *in vivo*.[34]

TABLE 3. COMPARISON OF 50 PER CENT INHIBITION OF DAO *in vitro* AND *in vivo* (values from Table 2)

Compounds	Quotient*
Iproniazid	1·3
Isoniazid	0·18
N^1-*iso*Nicotiynl-N^1 : N^2-di*iso*propyl	>52
*iso*Propylhydrazine	0·08
N^1 : N^2-Di*iso*propylhydrazine	1·0
N^1 : N^2-Di*iso*propylsemicarbazide	>66
Semicarbazide	0·01
Aminoguanidine	0·001

$$* \text{ Quotient} = \frac{\text{final concentration for 50 per cent inhibition } \textit{in vitro} \times 100}{\text{ED}_{50} \textit{ in vivo}}$$

A small quotient indicated a relatively stronger potency *in vitro* than *in vivo* and vice versa.

Inhibition of DAO *in vivo* by iproniazid lasts much shorter than that of MAO (Fig. 1).[8, 28, 35, 36] However, the inhibition of DAO similar to that on MAO[2, 28, 29] is hardly reversible by dialysis. Therefore the relatively quick recovery of DAO activity in rat intestine *in vivo* after administration of iproniazid might be due to rapid resynthesis of the blocked enzyme.

Iproniazid has some clinical actions which are less pronounced after application of isoniazid (e.g. antidepressive effect in mental disorders, hypotension, pain relief in angina pectoris). It has been assumed that these effects of iproniazid are related to MAO inhibition which *in vitro* as well as *in vivo* is more marked with iproniazid than with isoniazid. DAO has not been considered because the two compounds equally inhibit this enzyme *in vitro*. The present results show that iproniazid *in vivo* unlike *in vitro* is a markedly stronger DAO inhibitor than isoniazid*. Thus, DAO inhibition

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* Since this manuscript was submitted, the following further results have been obtained: Comparing inhibition of DAO and MAO in the same organ (cat kidney) DAO *in vivo* was about twice as much inhibited ($P < 0{\cdot}05$) by iproniazid as MOA (K. F. GEY, W. P. BURKARD and A. PLETSCHER, *Helv. Physiol. Acta*, in press (1960)).

could possibly also be of importance for some clinical and pharmacological effects of iproniazid.

Typical substrates the metabolism of which might be altered after DAO inhibition are agmatine, cadaverine, putrescine, spermine, spermidine[37] but in most species probably not histamine.[20, 38, 39, 40] Until the physiological function of these diamines is better known, no definite comments can be made on the possible consequences of DAO inhibition.

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