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DIFFERENTIATION OF MONOAMINE OXIDASE AND DIAMINE OXIDASE*

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Abstract—In kidney of cats pretreated with inhibitors of monoamine oxidase and diamine oxidase the activity of these enzymes was measured simultaneously, using typical cell fractions and substrates.

After iproniazid pretreatment inhibition of diamine oxidase occurred somewhat earlier and was of shorter duration than inhibition of monoamine oxidase (7 days for diamine oxidase and 21 days for monoamine oxidase).

Both amine oxidases may be inhibited independently *in vivo*: *d*, *l-trans*-phenyl-*cyclo*-propyl-amine and N-methyl-N-benzyl-2-propinylamine inhibit mainly monoamine oxidase, whereas aminoguanidine interferes only with diamine oxidase.

These results suggest that diamine oxidase is an enzyme with specific characteristics *in vivo*.

INTRODUCTION

MONOAMINE OXIDASE (MAO) and diamine oxidase (DAO) can be differentiated by their inhibitor spectrum *in vitro*[1] and by their intracellular distribution.[2] Characterization by inhibitors *in vivo*, however, is more difficult than *in vitro*. Thus, iproniazid† which *in vitro* inhibits DAO less than MAO[3, 4] reduces the activity of DAO *in vivo* to the same extent as that of MAO.[1, 4, 5] In contrast, semicarbazide and isoniazid,‡ typical and potent DAO inhibitors *in vitro*, interfere with this enzyme *in vivo* only moderately and less than iproniazid.[1, 5] The intracellular distribution of MAO and DAO is possibly not a good characteristic of the enzymes because DAO might represent a fraction of MAO eluted from mitochondria during conventional tissue homogenization and centrifugation.[6] Such an assumption is reasonable because within the same tissue the activity of DAO (present in the supernatant fraction) is, in general, markedly lower than that of MAO[7] (mainly associated with the mitochondrial fraction). Furthermore DAO has usually been demonstrated in organs rich in MAO but not in tissues devoid of the latter. Also both enzymes catalyze the same overall reaction[8] and have the same pH optima [4, 5] and attack monoamines as well as diamines though with some quantitative differences.[9, 10]

Differentiation of both amine oxidases by purification is difficult because it has been impossible to extract MAO from mitochrondria without loss of activity.[2] Differentiation by use of inhibitors *in vivo* has been of restricted value. On the one hand, MAO and DAO of various organs of rats were compared,[4] disregarding possible

* Partly published in a preliminary account.[1]

† N_2-*iso*propyl-*iso*nicotinic acid hydrazide, Marsilid^R.

‡ *iso*Nicotinic acid hydrazide, Rimifon^R.

pecularities of these tissues. On the other hand, MAO and DAO were measured in the same tissue, i.e. guinea pig liver, but DAO was determined by disappearance of histamine from total homogenate.[5] The greater part of histamine, however, is apparently not metabolized by DAO *in vitro* in liver of cattle, mice or cats, [9, 11–14], nor in intact animals including guinea pigs[15–17].

In order to distinguish DAO and MAO more clearly, cats were pretreated with several enzyme inhibitors followed by measurements of DAO and MAO activities in the same tissue using typical cell fractions and substrates for each enzyme. Cat kidney was selected because the activities of both oxidases are relatively high in this organ.

MATERIALS AND METHODS

Pretreatment of intact animals

Cats of both sexes varying in age and breed and weighing from 1·5 to 2·5 kg received aqueous solutions of the inhibitors i.p. after fasting for 24 hr. If not otherwise stated the inhibitors were applied in three to five doses (each dose in two to five animals) 16 hr before sacrificing by shooting. The kidneys were removed, freed from the capsule and pooled in ice-cold vessels, after being cut into small pieces. The pool was divided into two parts, one for DAO and the other for MAO. Untreated cats served as controls.

Measurement of MAO activity

Small pieces of kidney were homogenized in 4 vols. of 0·25 M sucrose in a Potter–Elvehjem homogenizer with a Teflon pestle. After a first slow centrifugation (30 min of 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 homogenous. All operations were performed at + 2 °C. MAO activity was determined in 0·067 M phosphate buffer at pH 7·2 with 0·009 M tyramine as substrate (final concentration). Oxygen uptake was measured at 37·5 °C under O_2 in Warburg manometers for 1 hr. The reaction was linear for at least 60 min.

TABLE 1. MEASUREMENT OF DIAMINE OXIDASE IN RAT INTESTINE BY WARBURG MANOMETRY AS WELL AS BY THE PHOTOMETRIC PROCEDURE OF HOLMSTEDT AND THAM[19]

The figures give $ED_{\bar{x}0}$ (doses for 50 per cent inhibition in μM/kg i.p.) ± fiducial limits for 95 per cent probability in parenthesis. Three to four doses of the inhibitors were applied to six rats each 4 hr before decapitation. The enzyme activity of supernatant (preparation corresponding to cat kidney) was measured by manometry with cadaverine as substrate as well as by photometry with putrescine as substrate (see Method).

Inhibitors	Method	
	Manometric	Photometric
Iproniazid	6 (5– 8)	6 (3– 18)
Phenelzine*	16 (9– 27)	18 (13– 25)
Isoniazid	49 (22–100)	91 (57–172)
Pivaloyl-benzylhydrazine†	147 (100–206)	202 (121–258)

* β-Phenylethyl hydrazine, Nardil^R.
† N_1-Trimethylacetyl-N_2 benzylhydrazine, Tersavid^R.

Measurement of DAO activity

Fifty per cent (w/v) homogenates in 0·067 M phosphate buffer at pH 7·2 (prepared at + 2 °C in a Servall Omni-Mixer with 16,000 rev/min for 45 sec) were saturated with octanol in order to inhibit MAO[18] and centrifuged at 29,000 g for 30 min. Incubation of the supernatant was carried out with 0·01 M putrescine (final concentration) at 37·5 °C under air in a metabolic shaker for 3 hr. The newly formed aldehyde was measured photometrically using *o*-amino-benzaldehyde.[19, 20] As shown for intestinal DAO this procedure gives results similar to those obtained with Warburg-manometry using cadaverine as substrate[4] (Table 1).

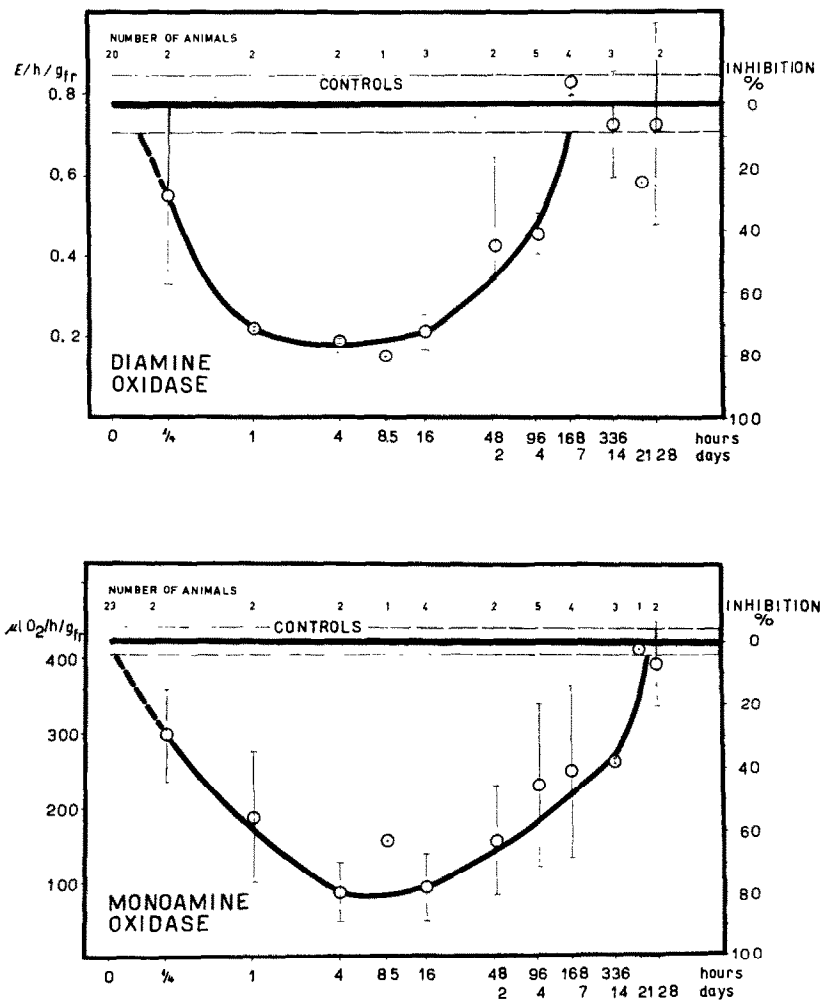


FIG. 1. Inhibition of diamine oxidase and monoamine oxidase of kidney in iproniazid-pretreated cats as function of time.

Abscissa: time in hours or days (on a logarithmic scale) after a single i.p. injection of 16·8 µM/kg (3 mg/kg) iproniazid.

Ordinates: left: absolute activity ($E_{1cm}^{4300Å}$/hr/g$_{fr}$ for DAO and µl O$_2$/hr/g$_{fr}$ for MAO).
right: per cent inhibition of the enzymes.

Mean values and standard error.

Calculations

The ED_{50} (dose for 50 per cent reduction of enzyme activity) was calculated by regression analysis.[21, 22] The fiducial limits[23] are given for 95 per cent probability.

RESULTS

Enzyme inhibition after pretreatment with iproniazid

A single i.p. dose of 3 mg/kg iproniazid (16·8 μM/kg) produced about 75–80 per cent inhibition of both enzymes in cat kidney. The maximal effect on DAO occurred

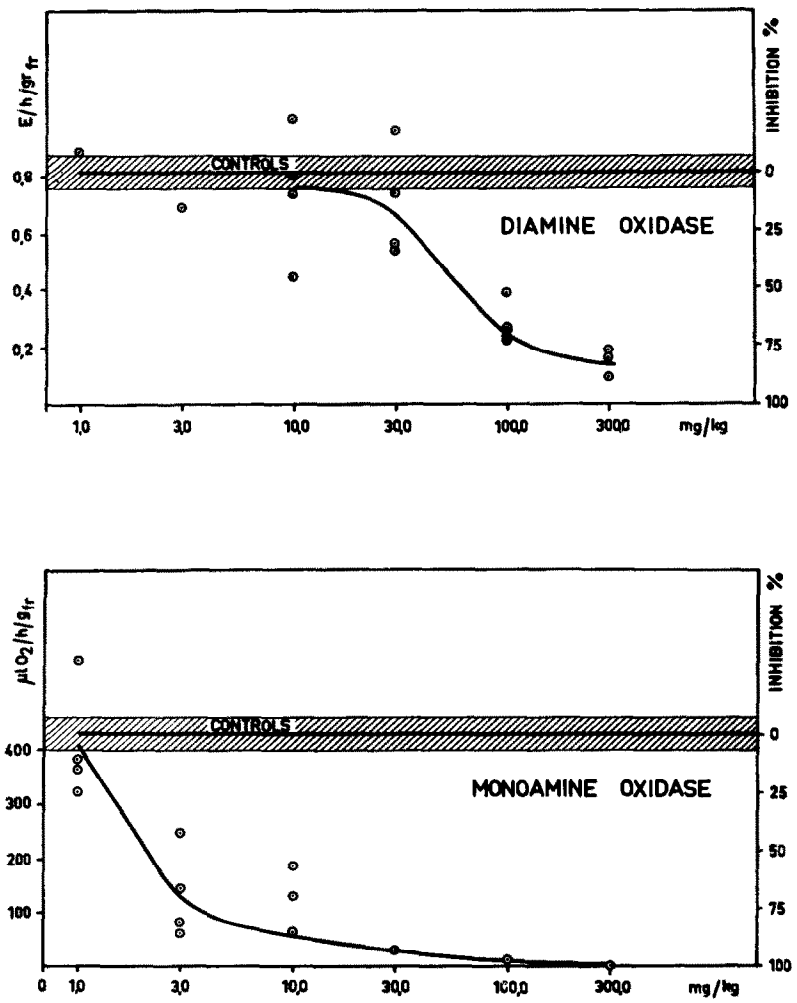


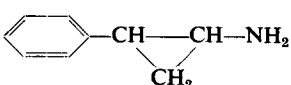
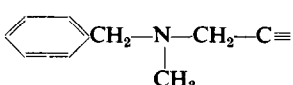
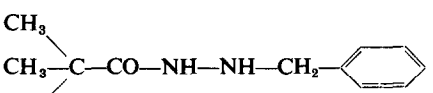
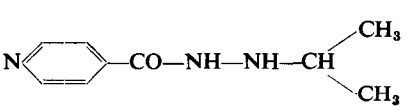
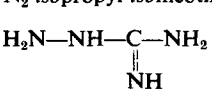
FIG. 2. Inhibition of diamine oxidase and monoamine oxidase in kidney of cats pretreated with N-methyl-N-benzyl-2-propinylamine

Abscissa: dose in mg/kg (m.w. 195·7) on a logarithmic scale i.p. 16 hr prior to sacrificing.
Ordinates: left: absolute activities.
right: per cent inhibition of the enzyme.
Each point represents a duplicate determination in one animal. Six cats served as controls.

after 1–16 hr, the enzyme activity was restored after 7 days. Maximal inhibition of MAO was observed after 4–16 hr, but the enzyme activity returned to control levels only after 21 days. Thus, DAO inhibition occurred somewhat earlier and was of longer duration than MAO inhibition (Fig. 1).

TABLE 2. COMPARISON OF DIAMINE OXIDASE AND MONOAMINE OXIDASE INHIBITION IN CAT KIDNEY BY VARIOUS COMPOUNDS

Activity of DAO and MAO was measured 16 hr after i.p. administration of the inhibitors in duplicates. Each inhibitor was compared to a group of untreated animals (4–6) sacrificed simultaneously. The degree of inhibition is expression by ED_{50} (dose for 50 per cent inhibition) ± fiducial limits (in parenthesis).

Inhibitor	Diamine oxidase		Monoamine oxidase	
	ED_{50} (μM/kg)	Number of cats	ED_{50} (μM/kg)	Number of cats
 *d, l-trans*-phenyl*cyclo*propyl-amine	> 168 (—)	1	3·6 (1·8–7·0)	11
 N-methyl-N-benzyl-2-propinylamine	450 (360–1450)	16	16·4 (14·8–19)	20
 N_1-pivaloyl-N_2-benzylhydrazine	20·2 (15·1–29·2)	20	2·6 (1–5·6)	10
 N_2-*iso*propyl-*iso*nicotinic acid hydrazide	3·5 (2·5–4·9)	8	6·9 (5–9·9)	8
 aminoguanidine	0·4 (0·2–0·6)	12	> 560 (—)	2

Quantitative comparison of DAO and MAO inhibition by various compounds

Comparison of the effect of various compounds shows that the activity of DAO can be reduced independently from that of MAO and vice versa. Thus, *d,l-trans*-phenyl*cyclo*propyl-amine* and N-methyl-N-benzyl-2-propinylamine,† two non-hydrazine compounds, inhibited MAO strongly but interfered only to a slight extent with DAO. Furthermore, N_1-pivaloyl-N_2-benzylhydrazine and iproniazid produced

* *trans*-Phenyl*cyclo*propyl-amine = Parnate^R.

† A 19120.

a marked inhibition of both DAO and MAO. Thereby, N_1-pivaloyl-N_2-benzyl-hydrazine affected MAO somewhat more than DAO, whereas the reverse was true for iproniazid. At last, aminoguanidine, also a hydrazine derivative, was the most potent DAO inhibitor, but had no measurable influence on MAO (Table 2, Fig. 2).

DISCUSSION

The above results confirm earlier findings that *in vivo* the time course of enzyme inhibition is different for MAO and DAO and that these two enzymes differ also in their inhibitor spectra.[4, 5] The present measurements are, however, more conclusive than the former because they were carried out with typical cell fractions of the same organ and with representative substrates for each oxidase. These differences between DAO and MAO suggest that DAO is not an artificial fraction of MAO but that MAO and DAO are distinct enzymes. The possibility cannot, however, be excluded that the two oxidases are isoenzymes.[24]

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