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THE MONOAMINE OXIDASE CATALYZED DEGRADATION OF PHENELZINE-1-^{14}C, AN IRREVERSIBLE INHIBITOR OF MONOAMINE OXIDASE—II

STUDIES *IN VIVO*

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Abstract—Rats receiving approx. 2·5 mg/kg of phenelzine-1-^{14}C by the intraperitoneal route were placed in metabolism cages and their urine collected for 24 hr. Phenylacetic-1-^{14}C acid was recovered as a major metabolite.

Pretreatment of the animals with tranylcypromine (10 mg/kg) or pargyline (100 mg/kg) prior to injection of radioactive phenelzine markedly reduced the urinary excretion of phenylacetic-1-^{14}C acid and the excretion of other unidentified metabolites appeared to increase. Inhibition of monoamine oxidase (MAO) with tranylcypromine or pargyline had no effect on the urinary excretion of an equimolar dose of phenylacetic-1-^{14}C acid administered by the same route. Therefore, pretreatment of rats with tranylcypromine or pargyline appears to have decreased the bioconversion of the injected phenelzine to phenylacetic acid. These results provide further evidence that phenelzine, in addition to being an irreversible inhibitor, is also a substrate of MAO in the intact animal.

PHENELZINE (β-phenylethylhydrazine; Nardil), like many derivatives of hydrazine, is a potent and irreversible inhibitor of monoamine oxidase (MAO; EC 1.4.3.4, monoamine: O_2 oxidoreductase deaminating).[1]

Leverett *et al.* reported finding radioactive phenylacetic acid conjugated with glycine (phenaceturic acid) in the urine of mice previously injected with phenelzine-1-^{14}C.[2] This observation, coupled with our demonstration that phenelzine can be dehydrazinated *in vitro* to phenylacetic acid via a reaction catalyzed by MAO,[3] prompted the present study of the effect of inhibition of MAO on the urinary excretion of phenelzine-1-^{14}C.

METHODS

Urine collection. After an i.p. injection of phenelzine-1-^{14}C–H_2SO_4, each male Sprague–Dawley rat (90–110 g) was placed in a metabolism cage designed for the separation of urine and feces. While in the cage, the animal had free access to water but not to food.

At the end of the period of collection, the animal was removed from the cage and the volume of urine in the collection receptacle recorded. The pH of the urine was

adjusted to a value of 1·0 with 6 N HCl. The sides of the metabolism cage were then washed with sufficient HCl (pH 1·0) to make a final volume of 25 ml in the urine collection receptacle.

A 50-μl specimen of the acidified diluted urine was removed and the radioactivity determined.

Extraction. Phenylacetic acid is excreted in most mammals as the glycine (phenaceturic acid) and glucuronic acid (phenacetylglucuronide) conjugates.[4] To free phenylacetic acid from its conjugates, it was necessary to subject the urine to a period of hydrolysis prior to extraction.

The acidified diluted urine from the collection receptacle was transferred to a 25-ml glass-stoppered graduated cylinder. The cylinder and contents were heated (95–99°) in a water bath for 5 hr.

After cooling, the hydrolyzed urine was poured from the cylinder into a pear-shaped 125-ml separatory funnel containing 8 g NaCl and 50 ml anhydrous ethyl ether. Twenty-five ml ether was then added to the cylinder, swirled and transferred to the separatory funnel. The total volume of ether used to extract the urine was, therefore, 75 ml.

Subsequent to shaking the funnel and allowing the phases to separate and settle, the aqueous phase was drawn off and discarded. The ether phase was collected and subsequently evaporated to a volume of 5 ml with the assistance of a stream of nitrogen.

A 50-μl quantity of the ether extract was withdrawn and the radioactivity ascertained.

Chromatography. Chromatography of ether extracts was carried out on sheets of microfilaments of glass impregnated with alumina gel or potassium silicate (ITLC type-A and type-S respectively; Gelman Instrument Co.) as described previously.[3]

Radioactivity measurements. The radioactivity of the various types of experimental samples was measured with a Mark I liquid scintillation computer (model 6860; Nuclear-Chicago Corp.). In addition to an efficiency correction (channels ratio method), all samples were corrected for background activity.

Fifty-μl quantities of the urine samples or ether extracts thereof were added to counting vials containing 20 ml of scintillation solution and counted. The scintillation solution used for counting ether extracts consisted of toluene as solvent and PPO (2,5-diphenyl-oxazole) in a concentration of 0·5% (w/v) as scintillator. When samples of urine were measured for radioactivity, the solution consisted of 4g PPO, 400 ml absolute ethanol and 600 ml toluene.

To determine the location of radioactivity on chromatograms, the area between the application origin and the solvent front was divided into twenty strips cut transversely to the direction of solvent movement. The strips (0·5 × 2·5 cm) were placed in vials containing 20 ml of 0·5% (w/v) PPO in toluene and the radioactivity ascertained.

Phenelzine-1-^{14}C-H_2SO_4 was a gift from Dr B. Dubnick and the Warner–Lambert Research Institute, Morris Plains, New Jersey The specific activity of the drug was 1·71 μc/mg and it was described as having a radiochemical purity of 99+ per cent.

Two samples of phenylacetic-1-^{14}C acid were obtained from New England Nuclear Corp. Their specific activities were 1·7 mc/m-mole and 8·4 mc/m-mole. The radiochemical purity of phenylacetic-1-^{14}C acid was greater than 96·5 per cent.

RESULTS

Effect of inhibitors of MAO on the urinary excretion of radioactivity after an injection of phenelzine-1-^{14}C. Pretreatment of rats with either tranylcypromine (10 mg/kg) or pargyline (100 mg/kg) caused a slight reduction in the amount of radioactivity found in the whole urine (Table 1). Whereas nearly 62 per cent of the radioactive label from injected phenelzine-1-^{14}C could be recovered in the 24-hr urine from control rats, about 54 per cent of the label could be recovered from those pretreated with tranylcypromine. The appearance of radioactivity in the urine was reduced to approx. 45 per cent of the amount injected as the result of pretreatment with pargyline.

With a hydrolysis period of 5 hr, nearly 86 per cent of the total urine radioactivity was extracted into ether (Table 1). If, however, the rats had been pretreated with tranylcypromine or pargyline, only about 55 per cent of the radioactivity present in the urine was extracted into ether.

TABLE 1. EFFECT OF INHIBITORS OF MAO ON THE URINARY EXCRETION OF PHENELZINE-1-^{14}C

Pretreatment		Animal no.	Urine vol. (ml)	Total urine		Ether extractable	
				dpm × 100	% inj.	dpm × 100	% urine
Control		1	10·0	7955	71·2	6513	81·9
		2	7·0	7850	70·3	6815	86·8
		3	5·0	6080	54·4	5340	87·9
		4	3·5	5765	51·6	4965	86·1
	Mean			6913	61·9	5908	85·7
Tranylcypromine		11	9·0	6540	58·5	2993	45·8
		12	5·5	6890	61·7	3810	55·2
		13	4·0	5950	53·2	3078	51·9
		14	1·5	4525	40·5	3168	70·1
	Mean			5976	53·5	3262	55·8
	% Control				86·4	55·2	65·1
Pargyline		21	8·0	6020	53·9	3794	63·1
		22	10·0	6500	58·2	3589	55·2
		23	1·5	2995	26·8	1390	46·3
		24	2·0	4680	41·9	2507	53·5
	Mean			5049	45·2	2820	54·5
	% Control				73·0	47·6	63·6

* Male Sprague–Dawley rats weighing 90–110 g were injected intraperitoneally with 0·25 mg phenelzine-1-^{14}C-H_2SO_4 1 hr after an i.p. injection of 1 ml distilled water (control group), 10 mg/kg of tranylcypromine sulfate (1 ml) or 100 mg/kg of pargyline hydrochloride (1 ml). After the injection of the radioactive phenelzine, each rat was placed in a separate metabolism cage and its urine collected for 24 hr. The 24-hr urine was diluted to 25 ml and the pH was adjusted to 1·0. The diluted urine was sampled for determining the total urine radioactivity prior to heating in a water bath for 5 hr. Subsequent to hydrolysis, the urine was extracted with 75 ml ether. The ether volume was reduced to 5 ml and a portion removed for determining the ether-extractable radioactivity. Each horizontal row of values in the table is from a single rat; animal no. identifies the urine from a particular animal.

It should be noted that the reduction in ether-extractable radioactivity resulting from pretreatment with pargyline or tranylcypromine was greater than the reduction in total urine radioactivity (Table 1). This finding argues against the possibility that the inhibitors of MAO affected the urinary excretion of injected phenelzine-1-^{14}C because of an alteration in kidney function.

Effect of inhibitors of MAO on the urinary excretion of radioactivity after an injection

of phenylacetic-1-^{14}C acid. Some animals were injected with an equimolar dose of phenylacetic-1-^{14}C acid instead of phenelzine. The results from these experiments are listed in Table 2. In all groups, ether-extractable radioactivity averaged approx. 87 per cent of the total present in the whole urine. This figure agrees well with the value of 85·7 per cent from the control rats receiving phenelzine-1-^{14}C (Table 1). The results with phenylacetic-1-^{14}C acid indicate that neither pargyline nor tranylcypromine affects the urinary excretion of phenylacetic acid.

TABLE 2. EFFECT OF INHIBITORS OF MAO ON THE URINARY EXCRETION OF PHENYLACETIC-1-^{14}C ACID*

Pretreatment	Animal no.	Urine vol. (ml)	Total urine		Ether extractable	
			dpm × 100	% inj.	dpm × 100	% urine
Control	1	11·0	2025	68·6	1749	86·2
	2	5·0	2805	95·1	2472	88·2
Tranylcypromine	11	12·5	1785	60·5	1529	85·8
	12	4·0	2650	89·8	2378	89·9
Pargyline	21	8·0	1600	57·0	1512	90·0
	22	3·5	1990	67·5	1678	84·3

* Except for the substitution of 0·146 mg phenylacetic-1-^{14}C acid for phenelzine-1-^{14}C, all procedures and conditions are the same as in Table 1.

Identification of phenylacetic-1-^{14}C acid in ether extract of urine from animals injected with phenelzine-1-^{14}C. Leverett *et al.* demonstrated the occurrence of phenylacetic acid in the urine of albino mice injected previously with phenelzine.[2] As shown in Figs. 1 and 2, the rat also excretes considerable phenylacetic-1-^{14}C acid after an injection of phenelzine-1-^{14}C.

In Fig. 1, a portion of the ether extract from control animal No. 2 (Table 1) was chromatographed for comparison with authentic phenylacetic acid. The extract exhibited a major elevation in the same area of the chromatogram (between 7 and 9·5 cm) as authentic phenylacetic-1-^{14}C acid. Although some authentic phenylacetic acid typically remained at the origin, a relatively greater amount of the radioactivity of the ether extract stayed at the point of application. As a consequence of this observation, it is clear that the ether extract contains metabolites of phenelzine other than phenylacetic acid.

In Fig. 2, a portion of the ether extract from control animal No. 4 (Table 1) was chromatographed for comparison with the chromatogram obtained with an equal amount of the ether extract from control experiment No. 1 (Table 2). The ether extract from the rat receiving phenelzine-1-^{14}C (control 4) showed two peaks in the same regions of the chromatogram as the peaks displayed by the ether extract from the rat receiving phenylacetic-1-^{14}C acid (control 1).

Peak B (Fig. 2) represents phenylacetic acid. This is true because: (a) the ratio of the dpm under peak B to the dpm under peak A plus peak B increased when authentic phenylacetic-1-^{14}C acid was added to the ether extract from control animal No. 4 (Table 1) prior to chromatographing; and (b) the majority of the radioactivity of the extract from control No. 2 (Table 1) was found to reside under peak B. As shown

previously (Fig. 1) with a different solvent system, most of the radioactivity in the extract from control No. 2 (Table 1) migrated to the same area of the chromatogram as authentic phenylacetic acid. No attempt was made to identify the material under peak A.

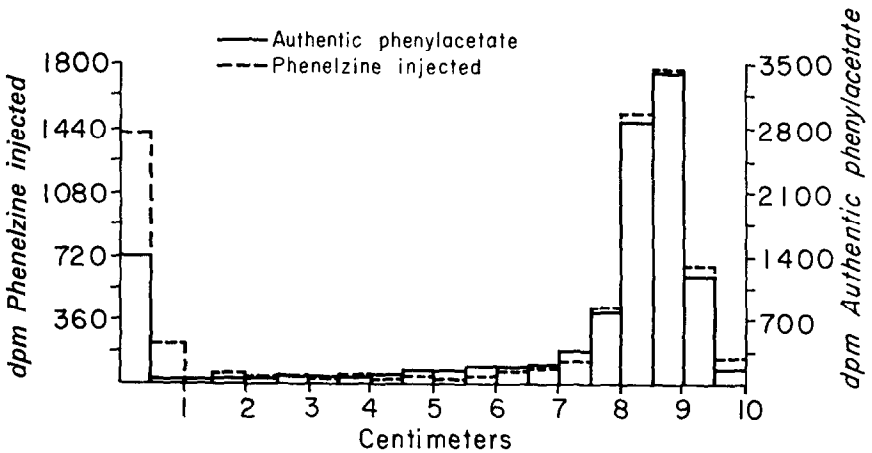

FIG. 1. Comparative radiohistograms of authentic phenylacetic-1-^{14}C acid and the ether extract of urine from a rat injected with phenelzine-1-^{14}C. Authentic phenylacetate or the ether extract was applied at the origin (0 cm) of the chromatographic sheet (type A). The solvent, chloroform:cyclohexane:glacial acetic acid (20:80:2), was allowed to advance past the origin for 10 cm. The sheet of chromatographic material was dried prior to dividing the area between 0 and 10 cm into twenty equal strips. The radioactivity of each strip was determined as described in Methods. The ratios of the components of the solvent system are volume ratios.

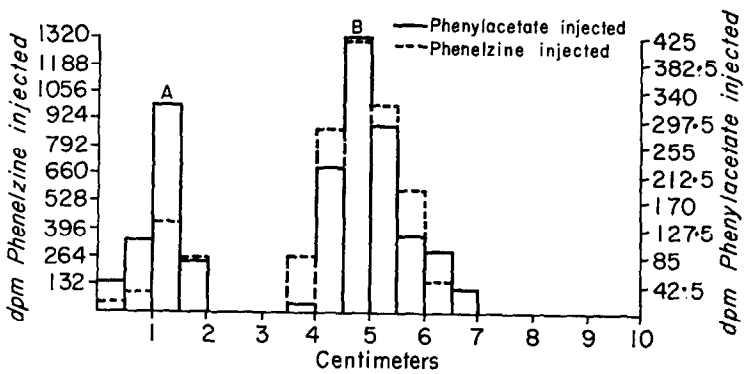

FIG. 2. Comparative radiohistograms of the ether extract of urine from a rat injected with phenelzine 1-^{14}C or phenylacetic-1-^{14}C acid. The ether extract was applied at the origin (0 cm) of the chromatographic sheet (type S). The solvent, benzene:*p*-dioxane:glacial acetic acid (90:25:4), was allowed to advance past the origin for 10 cm. The sheet of chromatographic material was dried prior to dividing the area between 0 and 10 cm into twenty equal strips. The radioactivity of each strip was determined as described in Methods. The ratios of the components of the solvent system are volume ratios.

Effect of inhibitors of MAO on the urinary excretion of phenylacetic-1-^{14}C acid after an injection of phenelzine-1-^{14}C or phenylacetic-1-^{14}C acid. To evaluate the effect of inhibitors of MAO on the biotransformation and excretion of phenelzine, equal portions of ether extracts from controls or pretreated animals were chromatographed and the dpm under peaks A and B totaled. It is valid to compare the combined activity inasmuch as, although it is not phenylacetate, peak A must represent a further metabolite of phenylacetate. Even though the amount of radioactivity under the two peaks was definitely reduced by pretreating the rats with pargyline or tranylcypromine, almost all of the ether-extractable radioactivity from all groups was found under peaks A and B (Table 3). In those instances where not quite all the radioactive label from phenelzine-1-^{14}C resided under peaks A and B (particularly tranylcypromine Nos. 11 and 13; pargyline Nos. 21 and 24), most of this activity was associated with the solvent front or with the strip immediately preceding or following the areas designated as being under peak A or B.

Results from animals injected with phenylacetic-1-^{14}C acid in place of phenelzine are also summarized in Table 3. Note that pargyline and tranylcypromine did not reduce the total activity under peaks A and B. All of the radioactive label from phenylacetate-1-^{14}C not found under peaks A and B was located in the strips immediately adjacent to one or both of the peaks.

In both the phenelzine- and the phenylacetate-injected animals, the radioactivity under peak B always exceeded that under peak A. However, from rat to rat there was considerable variability in the relative amount of activity under the two peaks (Table 3).

TABLE 3. CHROMATOGRAPHIC DISTRIBUTION OF ETHER EXTRACTS OF URINE FROM ANIMALS INJECTED WITH PHENELZINE-1-^{14}C OR PHENYLACETIC-1-^{14}C ACID

Treatment	Animal no.	Injection	dpm under peak A (× 100)	dpm under peak B (× 100)	dpm A + B (× 100)	% of ether extractable radioactivity
Control	1	Phenelzine-^{14}C	2657	3844	6501	99·8
	3		1015	4264	5279	98·9
	4		804	4164	4968	100·0
Tranylcypromine	11	Phenelzine-^{14}C	1155	1555	2710	90·5
	13		697	2086	2783	90·4
	14		798	2372	3170	100·0
Pargyline	21	Phenelzine-^{14}C	1530	1689	3219	84·8
	23		254	1102	1356	97·6
	24		601	1676	2277	90·8
Control	1	Phenylacetic	550	1195	1745	100·0
	2	acid-^{14}C	531	1939	2470	99·9
Tranylcypromine	11	Phenylacetic	535	993	1528	99·9
	12	acid-^{14}C	555	1556	2111	88·8
Pargyline	21	Phenylacetic	338	1101	1439	95·2
	22	acid-^{14}C	294	1392	1686	100·5

* Rats which had been injected intraperitoneally 1 hr previously with 1 ml distilled water (control group), 10 mg/kg of tranylcypromine sulfate (1 ml) or 100 mg/kg of pargyline hydrochloride (1 ml) received phenelzine-1-^{14}C—H_2SO_4 (0·25 mg) or phenylacetic-1-^{14}C acid (0·146 mg) by the same route. Their urine was collected and extracted with ether according to Methods. Ether extracts were chromatographed as described in Fig. 2. Peak A designates the area of the chromatogram between 0 and 2·5 cm and peak B includes the area between 3·5 and 7 cm (see Fig. 2). Per cent of ether-extractable radioactivity refers to dpm under A + B compared to the total ether-extractable radioactivity as listed in Tables 1 and 2.

DISCUSSION

Nearly 86 per cent of the radioactive label appearing in the 24-hr urine collected from rats injected with phenelzine-1-^{14}C could be extracted into ether (Table 1). The major metabolite of phenelzine present in the ether extract of the acidified and hydrolyzed urine chromatographed in a manner identical to authentic phenylacetic-1-^{14}C acid (Fig. 1). The formation of phenylacetate from phenelzine was also demonstrated by comparing the radiochromatographic distributions of ether extracts of urine from rats injected with phenelzine-1-^{14}C with those from rats injected with phenylacetic-1-^{14}C acid (Fig. 2). In either case, radioactivity concentrated in the same two areas of the chromatogram. The peak designated as "B" was shown to be phenylacetic acid (see Results), whereas the material(s) under peak "A" was not identified. The substance(s) under the unidentified peak may be conjugates of phenylacetate that failed to hydrolyze under the conditions employed. Chromatographic analysis of the urine from animals receiving phenelzine-1-^{14}C indicated, therefore, that essentially all of the ether-extractable radioactive material was either phenylacetic acid or a metabolite of phenylacetic acid (Table 3).

Approximately 14 per cent of the total radioactivity of the urine did not extract into ether (Table 1). Two explanations seem most apparent: (1) the extraction of phenylacetic acid and its conjugates was less than quantitative; or (2) phenelzine was, in part, excreted as products which would not extract into ether under the conditions employed. When an equimolar dose of phenylacetate-1-^{14}C was injected into the intact rat in lieu of phenelzine-1-^{14}C, approximately 87 per cent of the label appearing in the urine was extracted into ether (Table 2). This suggests, but certainly does not prove, that the first explanation above is correct and that phenelzine may have been converted entirely to phenylacetic acid *in vivo*.

Pretreatment of rats with tranylcypromine or pargyline decreased the 24-hr excretion of the radioactive label from phenelzine-1-^{14}C by 15–27 per cent but a more dramatic effect was the 45–52 per cent decrease in ether-extractable radioactivity (Table 1). In other words, pretreatment with one of the inhibitors of MAO markedly lowered the ratio of ether-extractable radioactivity/total urine radioactivity determined after an injection of phenelzine-1-^{14}C. Inhibition of MAO had no noticeable influence on the urinary excretion of injected phenylacetic-1-^{14}C acid. Thus, preadministration of pargyline or tranylcypromine did not affect the ratio of ether-extractable radioactivity/total urine radioactivity ascertained after injecting labeled phenylacetic acid (Table 2). These results taken together clearly indicate that inhibition of MAO prior to injection of phenelzine causes a shift away from phenylacetic acid formation with an increase in the formation and excretion of unidentified non-ether-extractable material.

Pretreatment of the rat with pargyline or tranylcypromine markedly reduced the amount of phenylacetic acid excreted after an injection of phenelzine (Table 3). However, inasmuch as the formation of phenylacetic acid was not completely eliminated, it must be able to form from phenelzine by processes not involving MAO or the pretreatment procedure was not sufficient to bring about total inhibition of MAO throughout the animal.

The pharmacological significance of the MAO pathway of inactivation of phenelzine has not been thoroughly investigated. Nevertheless, some pertinent observations have been made. Since phenelzine is both an inhibitor and substrate of MAO, one

might expect, provided that tachyphylaxis did not develop rapidly, the pharmacological responses from the drug to increase gradually upon repeated administration of small doses. Chessin *et al.*[1] observed a progressive increase in the vasopressor activity from repeated injection of phenelzine in the dog, and Clineschmidt and Horita[5] described a gradual increase in amplitude and duration of contraction of the nictitating membrane of the cat to successive administrations of phenelzine. The latter authors found that tachyphylaxis to the pressor action of phenelzine developed more rapidly in animals pretreated with nialamide, an inhibitor of MAO. As shown by Day and Rand,[6] cats pretreated with nialamide become tachyphylactic to injections of tyramine and phenylethylamine (both substrates of MAO) at a more rapid rate than control animals.

In 1965 Horita reported finding antimonoamine oxidase activity in liver supernatant fluid subsequent to an intraperitoneal injection of phenelzine into the intact rat, and treatment with other irreversible inhibitors of MAO before phenelzine greatly decreased the rate of disappearance of phenelzine activity from the liver supernatant fluid.[7] The results of the present investigation suggest that the decrease in rate of loss of phenelzine activity after inhibition of MAO resulted from a decrease in the formation of phenylacetic acid from phenelzine.

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