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FORMATION OF β-PHENYLETHYLAMINE FROM THE ANTIDEPRESSANT, β-PHENYLETHYLHYDRAZINE

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Abstract—To determine whether the monoamine oxidase inhibitor phenelzine was metabolized *in vivo* to produce β-phenylethylamine (PE) and *p*-hydroxy-β-phenylethylamine [*p*-tyramine (pTA)], a deuterated analogue, α,α,,β,β-^{2}H-phenelzine (d_4-phenelzine) was synthesized and injected i.p. into rats. In the first experiment, rat striata from d_4-phenelzine-treated rats were analyzed for the presence of d_4-PE and d_4-pTA at a time at which phenelzine was known to cause particularly large increases in striatal pTA. While d_4-PE was found to be present in these rat striata at a concentration equivalent to the endogenous PE, no d_4-pTA was present. The amounts of d_4-PE produced at various times after the i.p. injection of 50 mg/kg d_4-phenelzine were measured; at 1 hr post-injection, 371 ± 60, 1295 ± 682 and 1242 ± 394 ng/g (mean ± S.E.M.) d_4-PE were present in whole brain, liver and kidney. Rat urine collected for a 24-hr period after this treatment contained (mean ± S.E.M.) 88.5 ± 14.0 μg d_4-PE. These results clearly indicate that the antidepressant phenelzine was metabolized *in vivo* to produce the trace amine PE.

The antidepressant drug, β-phenylethylhydrazine (phenelzine) is a non-specific monoamine oxidase (MAO) inhibitor [1] which, in the rat, is excreted primarily as phenylacetic acid (PAA) [2, 3]. Though it has been proposed that oxidative dehydrazination of phenelzine is responsible for the production of PAA, some PAA may also be produced by oxidative deamination of β-phenylethylamine (PE), which in turn may have been formed from phenelzine. Several observations support this latter proposal. First, it has been reported that a similar MAO inhibitor, β-phenylisopropylhydrazine, is converted to an amine (β-phenylisopropylamine) in the rat [4], and second, two propargylamine MAO inhibitors, pargyline and deprenyl, are also converted to amines (benzylamine and amphetamine respectively) [5, 6]. In all of these cases, and this includes phenelzine, the chemical structure of the amine produced is present in the structure of the MAO inhibitor. Finally, Philips and Boulton [4] observed large increases in striatal PE and *p*-tyramine (pTA) levels following i.p. administration of phenelzine. Since it is known that pTA can be synthesized from PE by peripheral tissues of the rat [7], it seems possible that some of the large increase in striatal pTA levels could be due to synthesis of pTA from phenelzine via PE.

The following study was undertaken to determine whether phenelzine was converted to PE and pTA in the rat. We administered tetradeutero-phenelzine (d_4-phenelzine; α,α,β,β-$^2H_4$-β-phenylethylhydrazine) to rats and measured the amounts of deuterated PE, pTA and PAA present in their urine and several organs.

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MATERIALS AND METHODS

Materials. d_4-Phenelzine, d_2-PE, d_4-PE, d_4-pTA, d_4-mTA and d_5-PAA were synthesized as previously described [8–10] by B. A. Davis; d_9-PE was purchased from Merck, Sharp & Dohme, Pointe Claire-Dorval, Quebec. Organic solvents, HPLC grade, were obtained from Caledon Laboratories, Georgetown, Ontario; trifluoroethanol from PCR Research Chemicals Inc., Gainesville, FL; pentafluoropropionic anhydride from Chromatographic Specialties, Brockville, Ontario; PAA from the Aldrich Chemical Co., Milwaukee, WI; PE and 5-dimethylaminonaphthalene-1-sulfonyl chloride (dansyl chloride) from the Sigma Shemical Co., St. Louis, MO; and phenelzine from ICN Pharmaceuticals, Plainview, NY.

Drug treatments. Male albino Wistar rats (250–300 g, Charles River Laboratories, Montreal, Quebec) were used. They were housed in hanging wire cages and allowed free access to food and water. Phenelzine or d_4-phenelzine (50 or 100 mg/kg, dissolved in isotonic saline) was injected intraperitoneally at various times (10, 20, 40, 60 min, or 18 hr) before the animals were killed by cervical dislocation. The whole brain or the caudate nuclei, liver and kidneys were dissected out quickly, weighed and frozen on dry ice until they were homogenized in 0.1 N HCl.

Urine collection. Rats were housed for 24 hr in polycarbonate metabolic cages designed to collect urine free from fecal pellets (Canadian Lab. Supplies, Edmonton, Alta.). They were allowed free access to water but were not fed during this time. The urine collected in a container on the bottom of the cage plus the dried residues that were washed

into the collecting vessel were diluted to 20 ml and stored at −20°.

Mass spectrometric analyses. Mass spectrometric analyses were performed on an AEI MS902S double focussing instrument equipped with a VG ZAB console, DIGMID selected ion monitor (SIM), or a VG 70-70 F mass spectrometer (also equipped with DIGMID) and a VG 2000 (M8Z multispec) data system. Each instrument possessed a direct probe inlet used for amine analysis, and a GC inlet for acid analysis. The GCs were an HB 5700 with capillary injectors and a 50 m SGE 0V101 SCOT capillary column which was connected directly to the ion source with fused silica tubing. The carrier gas was helium (25–35 cm/sec flow rats), and the GCs were operated at 180° isothermally.

Amine analyses. The amounts of PE, mTA and pTA in the tissues or urine obtained from control and phenelzine-treated rats were measured following established high resolution mass spectrometric procedures [4, 11–13]. Briefly, known amounts (usually 40 ng) of the tetradeutero internal standards (d_4-PE, d_4mTA, d_4-pTA) were added to 1 ml aliquots of the urine or the homogenates of the tissue. The amines were converted to their dansyl derivatives with dansyl chloride and separated unidimensionally on two or three silica gel thin-layer plates. They were quantitated mass spectrometrically from the relative signals due to the molecular ions of the dansyl derivatives of the amine and its internal standard. The ions used were m/z 354.1402 and 358.1653 for dansyl PE and dansyl d_4-PE, respectively, m/z 603.1861 for *bis*-dansyl-mTA and *bis*-dansyl-pTA, and m/z 607.2113 for *bis*-dansyl-d_4-mTA and *bis*-dansyl-d_4-pTA.

In the first set of experiments, designed to determine whether d_4-phenelzine was converted to d_4-PE, d_4-mTA or d_4-pTA in the rat striatum, striata obtained from d_4-phenelzine-treated rats, were homogenized without the addition of d_4-internal standards, and then processed as described above. The ratios of the intensities of the molecular ions of the dansyl derivatives of d_4-PE and PE, of d_4-mTA and mTA, and of d_4-pTA and pTA were determined.

In the second set of experiments, the amounts of d_4-PE and PE in striata obtained from these d_4-phenelzine-treated rats were measured as described previously [7] by adding d_2-PE to half of the sample to quantify the amount of PE; then the amount of d_4-PE was determined from the ratio of the intensity of molecular ions of the dansyl derivatives d_4-PE and PE in the other half of the sample.

In the third set of experiments, the amounts of d_4-PE and PE in urine, liver and kidney obtained from d_4-phenelzine-treated rats were measured using a low resolution technique incorporating d_9-PE as the internal standard. Up to eight channels of the SIM were adjusted to detect the molecular ions of the proteo isotopomer, those of several deutero isotopomers including the d_4- and the d_9-ions, and a mass reference ion from heptacosafluorotri-*n*-butyl amine. In the case of PE, these were m/z values 354 (M+), 355 (d_1), 357 (d_3), 358 (d_4), 359 (d_5), 362 (d_8), 363 (d_9) and 364 (mass reference). For these analyses the mass spectrometer was operated at low resolution (1500–2000) so that formulas for correction of the abundances due to other isotopes (^{13}C, ^{15}N, ^{18}O, ^{34}S, etc.) could be used (see Beynon and Williams [14]).

Acid analyses. Acid metabolites were extracted and derivatized with trifluoroethanol and pentafluoropropionic acid anhydride as previously described [15]; d_5-PAA was used as the internal standard. For detection of the deuterated PAA metabolites of phenelzine, the SIM was operated to detect m/z values, 218 (M^+), 219, 221, 222, 223 (d_5), 226 and 214 (mass reference ion). The amounts of free (unconjugated) PAA were determined in 0.5-ml aliquots of rat urine. To determine the total (conjugated plus unconjugated) amount of PAA, 0.2 ml of concentrated HCl was added to 0.5 ml rat urine, and the conjugates were hydrolysed by heating the sample for 1 hr in a boiling water bath.

RESULTS

Conversion of d_4-phenelzine to d_4-amines in rat striatum. The ratios of the intensities of the molecular ions of the proteo and the tetradeutero species of the dansyl amine derivatives were compared (m/z 358 to 354 for PE and m/z 607 to 603 for the tyramines). Due to the natural distributions of the stable isotopes of C, N, O and S in the derivatized amines, the ratios of the intensities I_{358}/I_{354} and I_{607}/I_{603} are not zero in the spectra of the protonated standard

Table 1. Ratios of the intensities of the high mass (d_4) to low mass molecule ions of the dansyl derivatives of mTA, pTA and PE*.

	Intensity (I) ratios of		
	mTA (I_{607}/I_{603})	pTA (I_{607}/I_{603})	PE ($I_{358}I/_{354}$)
Standard compounds	0.017 ± 0.002 (N = 5)	0.029 ± 0.015 (N = 6)	0.003 ± 0.002 (N = 5)
Striatal samples*	0.026 ± 0.027 (N = 5)	0.074 ± 0.059 (N = 4)	1.04 ± 0.36† (N = 8)

* Values are expressed as mean ± S.E.M. The striatal samples were analysed 18 hr after injection of d_4-phenelzine (100 mg/kg). In other experiments, the concentrations of mTA, pTA and PE after this treatment were found to be (ng/g, mean ± S.E.M.) 16.7 ± 3.0, 314.4 ± 94.4 and 38.1 ± 6.9 respectively.

† Using Student's *t*-test, $P < 0.02$ compared to standard.

Table 2. Concentrations of some amines in rat striatum*

	mTa (ng/g)	pTa (ng/g)	PE (ng/g)	d_4-PE (ng/g)
Control	3.1 ± 0.3 (5)	17.7 ± 1.1 (5)	4.1 ± 1.2 (5)	0
d_4-Phenelzine (50 mg/kg)	32.0 ± 4.8 (4)	311.8 ± 31.1 (4)	32.4 ± 3.6 (4)	28.6 ± 6.1 (4)

* Values are (mean ± S.E.M., (N in brackets). d_4-Phenelzine was injected i.p. 18 hr before the animals were killed.

compounds. These small values are shown in Table 1. After injection of d_4-phenelzine, the intensity ratio I_{358}/I_{354} increased significantly from the standards showing the presence of d_4-PE in amounts equivalent to endogenous PE. The intensity ratio I_{607}/I_{603} seemed to increase slightly for pTA, but this increase was not significant ($P > 0.05$). The intensity ratio for mTA did not increase.

In other experiments (see Table 2), it was also observed that approximately equal amounts of d_4-PE and PE were present in rat striatum 18 hr after the i.p. administration of d_4-phenelzine (50 mg/kg). In all subsequent experiments, this dose of d_4-phenelzine was used, as some of the rats were killed by the larger dose (100 mg/kg). The increases in the concentrations of PE, mTA and pTA caused by these two different doses of d_4-phenelzine were similar (see Tables 1 and 2).

Purity of d_4-phenelzine, To ensure that the d_4-PE found in the rat striatum was due to systhesis from d_4-phenelzine rather than to any d_4-PE present in the drug as an impurity, samples (200 μg) of d_4-phenelzine were analysed for their d_4-PE content. In this amount of d_4-phenelzine there were 110 ± 2 ng (mean ± S.E.M., N = 4) of d_4-PE. This is equivalent to 0.06% of the weight of d_4-phenelzine. When 50 mg/kg d_4-phenelzine was injected into 250–300 g rats, then, 8–9 μg of d_4-PE was also injected. Though this is a small amount of d_4-PE, it is larger than the amount of d_4-PE found in the striatal tissue (about 2 ng in the striata from one rat). Rather than attempt the probably difficult task of determining whether this striatal d_4-PE came from the d_4-PE present in the d_4-drug or whether it was synthesized from the d_4-drug, we decided instead to determine the amounts of d_4-PE present in samples which were more likely to contain large amounts of d_4-PE, such as rat urine, liver, kidney and whole brain.

In addition, we made sure that the d_4-phenelzine which had been synthesized in this laboratory did not contain significant amounts of either PAA or deuterated (d_1,d_2)-PAA. Samples (10 mg) of d_4-phenelzine were analyzed for these acids. No measurable amounts of d_1-PAA or d_2-PAA were found, and only trace amounts of PAA (7 ng) were observed.

Urinary excretion of d_4-PE after d_4-phenelzine administration. The amounts of PE and d_4-PE excreted by control and by d_4-phenelzine-treated rats are shown in Table 3. The excretion of PE was increased by the d_4-phenelzine treatment; more importantly, however, a large quantity (88.5 μg) of d_4-PE was excreted after d_4-phenelzine administration. This amount (88.5 μg) was much larger than the amount of d_4-PE (8.1 μg) present as an impurity in the drug.

Conversion of d_4-phenelzine to d_4-PE in rat organs. As stated above, about 8 μg d_4-PE is present in the d_4-phenelzine injected into the rat. If this d_4-PE were to diffuse homogenously throughout the rat (250 g

Table 3. Urinary excretion of PE and d_4-PE by rats*

	PE (μg/24 hr)	d_4-PE (μg/24 hr)
Control	2.1 ± 0.3	0 ± 0
d_4-Phenelzine	29.3 ± 9.5	88.5 ± 14.0

* Values are mean ± S.E.M. (N = 4). d_4-Phenelzine (50 mg/kg) was injected i.p. immediately before the 24-hr urine collection.

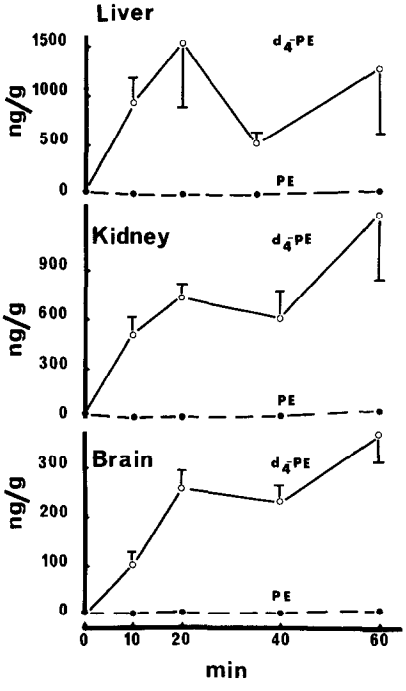

Fig. 1. Concentrations of d_4-PE and PE in liver, kidney and brain of d_4-phenelzine-treated (50 mg/kg) rats. Values are mean ± S.E.M. (N = or > 6). Key: (–○–○–), d_4-PE; and (–●–●–), PE.

body weight) without metabolic degradation, then a concentration of about 30 ng/g d_4-PE would be achieved. This concentration is most likely an overestimate, but it does give us a figure for comparative purposes. As can be seen in Fig. 1, the amounts of d_4-PE in the liver and kidney are well in excess of 30 ng/g at all time periods tested. In the brain, there was about 100 ng/g d_4-PE present 10 min after injection of d_4-phenelzine, but larger amounts were present later ($\simeq$ 250–300 ng/g). The amounts of PE present in the same samples are also shown; no marked changes in PE levels were observed.

In contrast to the marked increase in tissue levels of d_4-PE after administration of d_4-phenelzine, there was no observable increase in the amounts of d_1-PE or d_3-PE which might have conceivably contributed to the formation of d_1-PAA (see next section).

Urinary excretion of acid metabolites of d_4-phenelzine. The administration of d_4-phenelzine to rats did not change the excretion of free (unconjugated) PAA, but it did cause a large decrease in total (free + conjugated) PAA excretion (Table 4). The expected metabolite of d_4-phenelzine, d_2-PAA, was present in substantial amounts in urine, primarily as the conjugate. The total amount of d_2-PAA excreted within the first 24 hr was equivalent to 2.9% of the amount of d_4-phenelzine injected. Interestingly, large amounts of an unexpected metabolite, d_1-PAA, were also excreted, again mostly in the conjugated form.

DISCUSSION

The data presented in this paper clearly demonstrate that some of the MAO inhibitor d_4-phenelzine was converted to d_4-PE in the rat. While the concentration of PE attained in rat brain after injection of phenelzine is increased more than additively by prior injection of another MAO inhibitor [16], these data provide only indirect evidence for the conversion of phenelzine to PE. Moreover, the presence of small amounts of PE in non-dueterated phenelzine (the phenelzine which we had contained 0.028% PE) may have been responsible for the increased brain levels of PE in rats pretreated with another MAO inhibitor. Our data show that the amounts of d_4-PE present in urine, brain, kidney and liver obtained from rats injected with d_4-phenelzine were considerably greater than the small amount of d_4-PE present in the d_4-phenelzine that was injected. The data presented here do not delineate where or how d_4-phenelzine was metabolized to d_4-PE. The large amounts of d_4-PE present in the liver suggest that it may be a major site for such a conversion. d_4-PE produced in the liver conceivably could diffuse freely throughout the rat, and may, for example, be the source of d_4-PE in the brain.

Because striatal pTA levels are increased much more by administration of phenelzine than by other MAO inhibitors, it was previously thought that phenelzine might be metabolized to form pTA [4]. We demonstrated here that d_4-phenelzine, however, was not metabolized significantly to d_4-pTA in the rat striatum (see Table 1); thus, the large increase in striatal pTA must have arisen solely as a result of the inhibitory actions of the drug.

In addition to being an irreversible inhibitor of MAO, phenelzine is also a substrate for MAO. Pig brain MAO has been shown to oxidise phenelzine to produce phenylethylidenehydrazine [17]. In the rat, phenelzine has been shown to be degraded by MAO to phenylacetic acid [2,3], and it was suggested that a hydrazone (e.g. phenylethylidenehydrazine) may be an intermediate. Our work has shown that an alternate intermediate, namely phenylethylamine, may also be involved. In either case the finally excreted metabolite is phenylacetic acid which we have confirmed. If the administered phenelzine is labelled with deuterium atoms in the α and β positions of the alkyl chain, one would expect the excreted PAA to be labelled with two deuterium atoms (these remaining from the β positions of d_4-phenelzine). The unexpected finding that d_1-PAA was excreted may be due to the loss of a deuterium atom by keto-enol isomerism of duetero-phenylacetyldehyde which is produced as an intermediate, prior to the production of deutero-phenylacetic acid.

The major finding of this study, that the antidepressant phenalzine was converted to PE, may be of significance to the clinical actions of phenelzine. The antidepressant actions of phenelzine require chronic administration which results in much larger amounts of brain PE, probably because the amine produced is protected from degradation by MAO [16]. This PE may directly alter behavioural expression or it may possess indirect effects by affecting other monoamines.

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Table 4. Urinary excretion of some acid metabolites by rats*

		PAA (μg/24 hr)	d_1-PAA (μg/24 hr)	d_2-PAA (μg/24 hr)
Control	Free acids	96.8 ± 10.8	0.5 ± 0.2	0 ± 0
	Total acids	1480 ± 173	4 ± 3	0 ± 0
d_4-PEH	Free acids	98.9 ± 14.7	5.3 ± 2.6	11.1 ± 2.0
	Total acids	832 ± 218	278 ± 33	394 ± 42

* Values are mean ± S.E.M., N = 4. Rats were treated with d_4-phenelzine (d_4-PEH) (50 mg/kg, i.p.) immediately before the urine collection. Total acids were measured in urine samples that had been acid hydrolysed. The apparent values of d_1-PAA in controls are due to small errors in measurement of the ion intensities.

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