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POTENTIATION OF THE BIOCHEMICAL EFFECTS OF β-PHENYLETHYLHYDRAZINE BY DEUTERIUM SUBSTITUTION

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Abstract—The concentrations of dopamine (DA), *m*-tyramine (mTA), *p*-tyramine (pTA) and serotonin (5-HT) in the striata of rats 18 hr after the administration of three different doses (5, 50, or 100 mg/kg) of β-phenylethylhydrazine (phenelzine, PEH) were measured. These concentrations were compared to those following the administration of the same doses of 1,1,2,2-tetradeutero-PEH (d_4PEH). In general, PEH and d_4PEH caused dose-dependent increases in the levels of mTA, pTA and 5-HT. The lowest dose of d_4PEH caused greater increases than PEH in the levels of all four monoamines. The concentration of 5-HT was increased more by d_4PEH than PEH at all three doses. The inhibition of mitochondrial MAO obtained from rat striatum by PEH or d_4PEH *in vitro* revealed no differences. However, the inhibition of striatal MAO obtained from rats injected with d_4PEH was found to be greater than that from rats injected with PEH. It was concluded that deuteration of PEH potentiates its ability to inhibit MAO following its administration to the rat by slowing its degradation *in vivo*.

β-Phenylethylhydrazine sulfate (phenelzine, PEH) is used clinically as an antidepressant [1]. It is a non-specific monoamine oxidase (MAO) inhibitor [2] which increases the concentrations of various monoamines in the rat brain [3–7]. In a recent study, we synthesized 1,1,2,2-tetradeutero-phenelzine (d_4PEH), administered it to rats, and noticed that it had more pronounced behavioural effects on the animals than unsubstituted PEH.† PEH is a substrate as well as an inhibitor of MAO [8–11]. PEH itself is an irreversible inhibitor of MAO [8]. MAO isolated from rat liver, bovine kidney, and pig brain oxidizes PEH to form phenylacetaldehyde hydrazone; during this process, the α-hydrogen adjacent to the hydrazine moiety is removed [8–10]. It has also been shown that PEH administered to a rat is oxidized further to form phenylacetic acid [11, 12]. Since a carbon–deuterium bond is more difficult to break than a carbon–hydrogen bond, the replacement of the α-hydrogens of PEH with deuterium might retard its biological inactivation; hence, d_4PEH might appear to be a more effective MAO inhibitor than unsubstituted PEH. We have, therefore, compared the abilities of these two drugs to inhibit striatal MAO, first by measuring the elevation of the concentrations of several endogenous monoamines in the rat striatum, and second by determining striatal MAO activity *in vitro* and *ex vivo*.

MATERIALS AND METHODS

To ensure high sensitivity (i.e. low blank values in the mass spectrometric analyses), all solvents were redistilled reagent grade chemicals and the glassware was cleaned in chromic acid (for details see Refs. 5 and 13–15). β-Phenylethylhydrazine sulfate was purchased from ICN Pharmaceuticals (Plainview, NY, U.S.A.).

The internal standards for mass spectrometry: 1,1,2,2-$^2H_4$-2-(4-hydroxyphenyl)-ethylamine (deuterated *para*-tyramine, d_4pTA) and 1,1,2,2-$^2H_4$-2-(3-hydroxyphenyl)-ethylamine (deuterated *meta*-tyramine, d_4mTA) were synthesized as described previously [16]. Radiochemicals were purchased from the New England Nuclear Corp. (Boston, MA, U.S.A.).

Synthesis of deuterated phenelzine (d_4PEH). Phenylacetonitrile was first converted into 1,1-dideuterophenylacetic acid by refluxing overnight in 10% sodium deuteroxide in deuterium oxide (D_2O) and then into the ethyl ester under acidic conditions. The ester was reduced with lithium aluminum deuteride to 1,1,2,2-tetradeutero-2-phenylethanol which was converted to 1-chloro-1,1,2,2-tetradeutero-2-phenylethane with thionyl chloride. The chloride was refluxed with hydrazine and the 1,1,2,2-tetradeutero-2-phenylethylhydrazine (d_4PEH) precipitated as the sulfate salt. It was recrystallized from methanol/ether to give a white solid (m.p. 167–168°). A low resolution mass spectrum exhibited no molecular ion, but major peaks were observed at *m/z* 31, 47, 93 and 109. The isotopic purity was determined from the spectrum of the acetone (1-dimethylamino-5-napthalene sulphonyl)-2-phenylethyl hydrazone. The d_4PEH was found to be 91% tetradeutero and 9% trideutero.

Drug treatments. Male albine Wistar rats (180–250 g, Charles River Laboratories Canada, Montreal, Quebec) were used in all experiments. They were maintained in hanging wire cages and allowed

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† C. T. Dourish, K. Dewar, L. E. Dyck and A. A. Boulton, unpublished data.

access to food and water *ad lib*. PEH and d_4PEH, dissolved in isotonic saline, were injected i.p. (5, 50 or 100 mg/kg) 18 hr before the animals were killed. Control rats received saline.

After the rats were killed by cervical dislocation, the striata were removed quickly, weighed, and frozen on dry ice until they were homogenized.

Amine analyses. The amounts of mTA and pTA in the tissues obtained from control and drug-treated rats were measured following established mass spectrometric procedures [5, 13–15]. Briefly, the tissues were homogenized in 0.1 N HCl to which known amounts (usually 40 ng) of the tetradeutero internal standards (d_4mTA, d_4pTA) had been added. 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* 603.1861 for bis-dansyl-mTA and bis-dansyl-pTA and *m/z* 607.2113 for bis-dansyl-d_4mTA and bis-dansyl-d_4pTA.

The amounts of serotonin (5-HT) and dopamine (DA) in striatal tissue, obtained from other control and drug-treated rats, were measured by a procedure involving high pressure liquid chromatographic separation and electrochemical detection [17]. Isoprenaline was used as the internal standard.

Estimation of monoamine oxidase activity. The activities of mitochondrial membrane MAO from control and drug-treated rats (5 mg/kg, 18 hr) were estimated *ex vivo*. Striatal tissues were homogenized in 0.32 M sucrose, and the crude mitochondria were obtained by differential centrifugation. The mitochondrial membrane fragments, which were prepared by homogenization of the mitochondria in 0.02 M phosphate buffer (pH 7.5), were used for the enzyme assays. MAO activity was determined radioenzymatically as previously described [18] using [1-^{14}C]*p*-tyramine (1×10^{-4} M), 2-[1-^{14}C]phenylethylamine (2.5×10^{-5} M) and 5-[2-^{14}C]hydroxytryptamine (1×10^{-4} M) as substrates. In addition, inhibition *in vitro* of the MAO activity of tissue obtained from saline-treated rats was measured by addition of various amounts of PEH or d_4PEH.

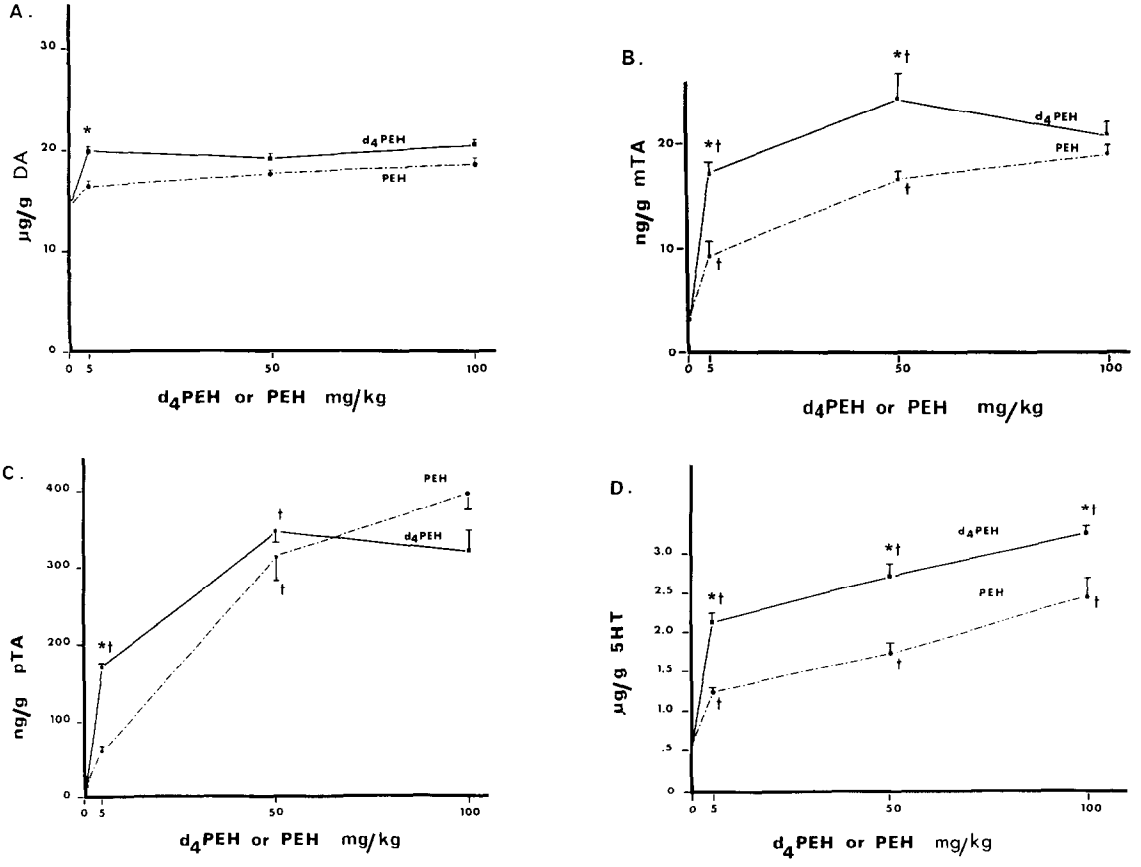

Fig. 1. Effects of PEH and d_4PEH on the concentrations of DA, mTA, pTA and 5-HT in rat striatum. Values are means ± S.E.M. Control values were: DA, 14.9 ± 0.5 μg/g (N = 12); mTA, 3.1 ± 0.3 ng/g (N = 5); pTA, 17.7 ± 1.1 ng/g (N = 5); and 5-HT 0.66 ± 0.05 μg/g (N = 4). The values were subjected to one-way analysis of variance, and the significance between means was determined by the Newman–Keuls method [19]. Key: (*) This value for d_4PEH is different from the value for the equivalent dose of PEH. (†) This value is different from the same drug at a lower dose ($P < 0.05$). Note for DA that all values (except for 5 mg/kg PEH) are different from the control value (but not necessarily larger than the immediately lower dose value).

RESULTS

Effects of PEH and d₄PEH on the concentrations of several striatal monoamines. The effects of intraperitoneally administered PEH and d₄PEH on the striatal concentrations of DA, 5-HT, mTA and pTA were compared (see Fig. 1, A–D). The concentration of DA in the rat striatum was increased significantly above control levels by the administration of PEH and d₄PEH. No dose dependencies were observed with either drug. A significantly greater increase in the DA concentration, however, was observed with a 5 mg/kg dose of d₄PEH compared to the same dose of PEH (Fig. 1A). Administration of 5 and 50 mg/kg doses of PEH and d₄PEH caused mTA and pTA concentrations in the rat striatum to increase with dose (Fig. 1, B and C). No further increase was observed after administration of doses of 100 mg/kg. d₄PEH was significantly more potent than PEH in inhibiting oxidation of mTA and pTA at the lower doses (5 and 50 mg/kg for mTA; 5 mg/kg for pTA). The concentration of 5-HT increased with each larger dose of either drug (Fig. 1D), and d₄PEH was significantly more potent than PEH in inhibiting oxidation of 5-HT at all doses.

Effect of PEH and d₄PEH on rat striatal MAO activity. The inhibition of striatal mitochondrial MAO by PEH and d₄PEH *in vitro* is shown in Fig. 2. The inhibitory activities of PEH and its deuterium substituted analogue, d₄PEH, on the oxidation of 5-HT and β-phenylethylamine (PE) appeared quite similar.

In contrast to these *in vitro* observations, when the rats were injected intraperitoneally with 5 mg/kg PEH or d₄PEH and the striatal MAO activity was subsequently estimated radioenzymatically, a significant difference in MAO activity was found (see Table 1). While both PEH and d₄PEH significantly inhibited the activity of rat striatal MAO towards 5-HT, pTA and PE, d₄PEH exhibited significantly higher inhibitory activity than did PEH in this *ex vivo* study. This difference was observed with all three amine substrates.

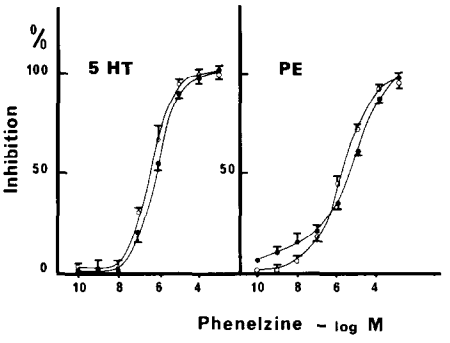

Fig. 2. Effects of PEH and d₄PEH on rat striatal MAO activity towards radiolabeled 5-HT and β-phenylethylamine. The labeled substrates were incubated with rat striatal mitochondrial MAO (30 mg protein/assay) in the presence of different concentrations of PEH (○—○) and d₄PEH(●—●). Values are means ± S.E.M.; N = 3.

DISCUSSION

It has been shown that PEH increases the brain concentrations of 5-HT, noradrenaline [3, 4], PE [5], DA, mTA and pTA [4–7]. Our present results concur with these findings. The concentrations of mTA, pTA and 5-HT increased markedly after even the lowest doses (5 mg/kg) of PEH or d₄PEH and were generally dose dependent (Fig. 1, B–D). A comparison of the striatal concentrations of PE following administration of PEH or d₄PEH to the rat will be reported elsewhere because, in other studies in progress, we observed the complicating factor that intraperitoneal PEH can be converted to PE; therefore, any increase in PE levels following PEH administration cannot be attributed solely to inhibition of the degradation of PE by MAO. A comparison of the abilities of PEH and d₄PEH to increase the striatal levels of DA, mTA, pTA and 5-HT revealed that d₄PEH generally caused greater effects. Thus, for DA, mTA and pTA, d₄PEH was

Table 1. Effect of intraperitoneal administration of PEH and d₄PEH on rat striatal monoamine oxidase activity*

	Specific MAO activity [nmoles·(mg protein)$^{-1}$·min^{-1}]		
	5-HT	pTA	PE
Control	1.35 ± 0.10 (100%)	1.96 ± 0.18 (100%)	1.02 ± 0.09 (100%)
PEH	0.60 ± 0.02† (44.4%)	1.04 ± 0.04† (52.8%)	0.80 ± 0.09 (78.4%)
d₄PEH	0.24 ± 0.05†‡ (17.8%)	0.40 ± 0.09†‡ (20.3%)	0.32 ± 0.04†‡ (31.4%)
F[2,9] $P < 0.05$	69.4	43.4	19.6

* Results are expressed as the mean values ± S.E.M. of four animals in each group and the percent of control activity is shown in parentheses. The F values were determined from one-way analysis of variance of each set. The rats were injected i.p. with PEH (5 mg/kg) or d₄PEH (5 mg/kg) dissolved in saline. Brain striata were dissected 18 hr after the injection. Control animals were treated with saline. Differences between means were determined from application of the Newman–Keuls method [19].

† Significantly different from controls ($P < 0.05$).

‡ Significantly different from PEH values ($P < 0.05$).

more potent at the lower doses, whereas with respect to striatal 5-HT concentrations d_4PEH was more potent than PEH at all doses.

Our data regarding the ability of PEH to inhibit striatal MAO *in vitro* agree with Christmas *et al.* [3], who reported that 10^{-6} M PEH produced a 50% inhibition of the oxidation of 5 HT, pTA and benzylamine by rat brain MAO. In agreement with these authors, we also observed a differential effect toward different types of substrate when the drug was administered to the animal rather than added *in vitro*. It is also interesting to note that the deaminations of pTA and mTA were inhibited similarly by clorgyline and deprenyl *in vitro*, but a differential effect was observed when the inhibitors were applied *in vivo* [20].

Since d_4PEH was not more potent than PEH in inhibiting MAO *in vitro*, the mechanism of inhibition of MAO by the two analogues is probably the same. After injection of the two drugs into rats, however, the specific MAO activity of striata obtained from rats which had received the deuterated drug was reduced to an extent greater than that from rats which had received PEH. This observation was true whether a type A or type B substrate was employed. To the best of our knowledge, no other reports of the potentiation of the efficacy of an MAO inhibitor by substituting deuterium atoms for hydrogen atoms exist, although it is known that substitution of the α-hydrogens of various arylalkyl amines with deuterium reduces their enzymatic deamination by MAO [21, 22]. The oxidation of PEH by MAO appears to be a quantitatively significant pathway [8–12]; hence, it seems likely that deuterium substitution of phenelzine enhances its central potency by slowing its peripheral inactivation, thus initially producing higher levels in the brain.

In order to show that more d_4PEH than PEH was present in the brain after parenteral administration of these drugs, it is necessary to measure their levels at a time shortly after injection, when the highest levels of the drug reach the brain. Twenty minutes after injection of the drugs, 1.25 times more d_4PEH than PEH was present in the brain (unpublished observations); this finding most likely reflects differences in the rates of peripheral degradation and transport into the brain, but it is not indicative of differences in the degree of MAO inhibition in the brain. The concentration of PEH, or d_4PEH, does not correlate directly with MAO inhibition. When PEH inhibits MAO, it is no longer measurable as PEH either because it is metabolized to some other compound or because it is irreversibly bound to the enzyme [8–12]; in both cases, a decrease in the concentration of PEH corresponds to inhibition of MAO. We also attempted to measure the concentrations of d_4PEH and PEH in the brain 5 hr after injection; however, the small amounts of drug left in the brain were not reliably measurable. At such a late time, the decreased levels of PEH were presumably due to transport out of the brain, degradation of the drug, and irreversible binding of the drug to MAO. As stated previously, the concentration of PEH does not correspond to MAO inhibition; therefore, we have not pursued this matter.

In summary, we have shown that, in the rat, substitution of the alkyl hydrogens of PEH with deuterium atoms led to an enhanced ability of the drug to increase striatal DA, mTA, pTA and 5-HT levels and an enhanced ability to inhibit striatal MAO after intraperitoneal administration.

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