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SHORT COMMUNICATIONS

Effect of deuterium substitution on the penetration of β-phenylethylhydrazine into the rat brain

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β-Phenylethylhydrazine (phenelzine, PLZ) is a potent irreversible inhibitor of monoamine oxidase (MAO) both *in vivo* and *in vitro* [1]. The chemical structure of PLZ is similar to the trace amine β-phenylethylamine (Fig. 1); apparently its similarity to a monoamine is sufficient for it to be a substrate of MAO [2]. Tipton and Spires [3] demonstrated that PLZ is oxidized by MAO to its hydrazone derivative, phenylethylidenehydrazine, in a reaction that is analogous to that of amine oxidation [4].

Stereospecific deuterium labelling on the α-carbon of certain biogenic amines enhances the effects of these drugs in various experimental paradigms [5–8]. The basis for this facilitation appears to be related to an attenuation of the deamination of the deuterated drug by MAO [7–10] resulting in larger quantities of the deuterated drug reaching the brain [10–12]. Similarly, deuterium substitution on the α,α,β,β-positions of PLZ enhances the behavioural [13] and biochemical potency [14] of this drug *in vivo*. Consequently, it has been postulated that deuterated PLZ is more resistant to deamination by MAO than its non-deuterated analog, thus allowing more of the systemically administered deuterated drug to gain access into the brain. To assess this hypothesis, we injected an equimolar mixture of deuterated and non-deuterated PLZ intraperitoneally into rats and measured the ratio of PLZ and [$^2H_4$]PLZ in the brain. Furthermore, we examined whether pretreatment with an MAO inhibitor would influence the proportion of PLZ and deuterated PLZ reaching and persisting in the brain.

Methods

Pargyline HCl was purchased from the Sigma Chemical Co., St. Louis, MO; phenelzine sulfate was purchased from I.C.N. Pharmaceuticals Inc., Plainview, NY, and α,α,β,β-[$^2H_4$]phenelzine sulfate was synthesized as described previously [13]. Adult male Wistar rats (200–250 g, Charles River Canada, Montreal, Quebec) were housed in hanging wire cages on a 12-hr dark/light cycle and allowed free access to food and water. All drugs were dissolved in distilled water and injected intraperitoneally. In the first set of experiments, animals were treated with an equimolar mixture of PLZ and α,α,β,β-[$^2H_4$]PLZ (50 mg/kg of each) and killed 0.25, 1, 2, and 5 hr after drug treatment. In the next set of experiments, the animals received similar drug treatment as above; however, they were pretreated with pargyline (50 mg/kg) 16 hr prior to being killed. Rats were killed by cervical dislocation, and their brains were removed and homogenized in 4 ml of 0.05 N perchloric acid. Phenelzine and α,α,β,β-[$^2H_4$]PLZ were converted to their dansyl-acetone-imine derivatives by the addition of 4 ml dansyl chloride reagent (10 mg/ml in acetone). The dansyl derivatives were extracted into benzene (2 ml × 2) and then separated unidimensionally on two silica gel thin-layer plates using chloroform : ethyl acetate (4 : 1, v/v) and carbon tetrachloride : triethylamine (5 : 1, v/v) as the solvent systems respectively. The ratios of the dansyl-acetone-imine derivatives of PLZ and α,α,β,β-[$^2H_4$]PLZ was calculated mass spectrometrically according to the procedure described by Dyck [15]. A check solution, an aliquot of the drug solution made up for injection containing 250 μg each of deuterated and non-deuterated PLZ, was analysed in a manner similar to that of the tissue sample. The check solution provided a measure of the ratio of [$^2H_4$]PLZ to PLZ in the injected drug solution and, therefore, was used as a correction factor for the tissue samples.

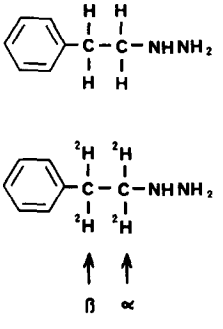

Fig. 1. Structures of [$^2H_4$]phenelzine and phenelzine.

Results and discussion

The ratios of the amounts of [$^2H_4$]PLZ and PLZ present in the brain at various time intervals after injection of an equimolar mixture of α,α,β,β-[$^2H_4$]PLZ and PLZ were compared to the corresponding ratios after pretreatment with the MAO inhibitor pargyline (50 mg/kg). The data were analysed by two-way analysis of variance followed by *a priori* *t*-tests. The ratios of the amounts of [$^2H_4$]PLZ to PLZ present in the brain 0.25, 1 and 2 hr after injection of an equimolar mixture of these drugs were 1.32 ± 0.01, 1.36 ± 0.05, and 1.39 ± 0.06 (mean ± SEM, N = 8). At these time periods, more [$^2H_4$]PLZ than PLZ penetrated and persisted in the brain. When the animals were pretreated with pargyline, the ratios of the amounts of [$^2H_4$]PLZ and PLZ present in the brain at these times were decreased significantly (1.04 ± 0.01, 1.06 ± 0.01 and 1.04 ± 0.02, N = 6, respectively) (Fig. 2). Thus, with pargyline pretreatment the amount of [$^2H_4$]PLZ in the brain was no longer greater than the amount of PLZ. Similarly, equal amounts of [$^2H_4$]PLZ and PLZ were present in brain 5 hr after injection of an equimolar mixture of these drugs in the presence or absence of pargyline.

Substitution of deuterium for hydrogen on the α,α,β,β-positions of PLZ has been shown to increase the biochemical [14] and behavioural [13] potency of this drug *in vivo*. Furthermore, deuterated PLZ reduces striatal MAO activity to a greater extent than the non-deuterated drug [14], suggesting that deuterium substitution facilitates the potency of PLZ by increasing its ability to inhibit MAO. The potencies of deuterated PLZ and non-deuterated PLZ in inhibiting MAO *in vitro*, however, are comparable [14]. As a consequence, the increased effectiveness of α,α,β,β-[$^2H_4$]PLZ as an inhibitor of cerebral MAO *in vivo* cannot be attributed to a direct action of this drug on the enzyme itself; rather, deuteration enhances the potency of cerebral

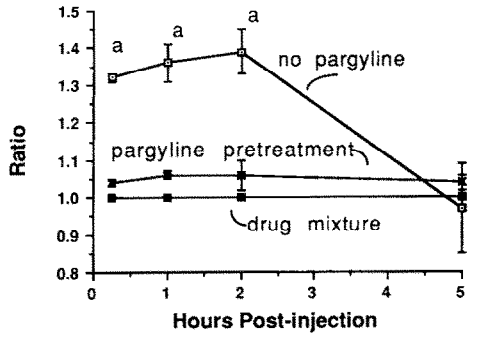

Fig. 2. Ratio of [$^2H_4$]phenelzine to phenelzine levels in rat brain at various times after injection of 50 mg/kg each of phenelzine and [$^2H_4$]phenelzine. Key: (a) $P < 0.05$ comparing the ratios of the drugs at a particular time period in rats with or without pargyline (50 mg/kg, 16 hr prior to rats being killed).

PLZ indirectly. Stereospecific deuterium substitution of various arylalkyl amines has been found to reduce their enzymatic deamination by MAO [7–10]. Intraperitoneally administered PLZ is subjected to extensive catabolism by the liver, primarily by MAO, before it is circulated throughout the body [16]. [$^2H_4$]PLZ appears to be protected from such catabolism on its first pass through the liver; thus, more [$^2H_4$]PLZ is available to enter the brain. This suggestion is supported by the present finding that pretreatment with an MAO inhibitor abolished the effect of deuteration. Similarly, at later time periods, the amount of [$^2H_4$]PLZ in the brain was no longer greater than the amount of PLZ, because of continuing metabolism of the drugs by the liver and other organs.

In summary, previous studies have shown that deuterium substitution in the $\alpha,\alpha,\beta,\beta$-positions of PLZ increases the behavioural and neurochemical potency of this drug, presumably through an attenuation of the metabolic inactivation of [$^2H_4$]PLZ by MAO. In this paper, we show that the concentration of [$^2H_4$]PLZ transported into the brain was significantly greater than that of the non-deuterated drug.

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Putative binding site(s) of 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) on protein kinase C

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Protein phosphorylation is a major mechanism controlling intracellular events in mammalian tissues, as related to external physiological stimuli [1, 2]. Protein kinase C, an enzyme activated by calcium ion, phosphatidylserine and diacylglycerol, phosphorylates various protein substrates and relays transmembrane signalling in diverse Ca^{2+}-dependent cellular responses [3, 4]. As the manipulation of protein kinase by synthetic compounds should have profound effects on various cell functions, we developed selective inhibitors of protein kinase C, 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7) [5]. Studies using H-7 suggested that protein kinase C may play an important regulatory role in the function of various tissues and cells, including human platelets [6] and acinar cells of the pancreas [7]. However, the precise binding site of H-7 on protein kinase C had to be determined. As H-7 inhibits phosphotransferase activities, competitively with ATP [5], it is desirable to use reagents that covalently modify the