Communications to the Editor

Interaction of Monoamine Oxidase B with Analogues of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine Derived from Prodine-Type Analgesics

Sir:

A recent article by Baum[1] suggests that analogues of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **4**), which can appear as side products in the synthesis of analogues of the reverse esters of meperidine (MPPP, **1**; see Scheme I), may be potential neurotoxins. Particular attention was given to 1-(2-phenylethyl)-4-phenyl-1,2,3,6-tetrahydropyridine (PEPTP, **6**), which is a possible contaminant of the illicitly produced analgesic 1-(2-phenylethyl)-4-phenyl-4-propionoxypiperidine (PEPPP, **3**) and considered by Langston to be a neurotoxin.

We now report that 1,3-dimethyl-PTP (**7**) and 1,5-dimethyl-PTP (**5**), derived from α- or β-prodine (**2**), and PEPTP (**6**) from PEPPP (**3**) are not readily metabolized by preparations of pure human liver monoamine oxidase B:antibody complex.[2]

For our studies MPTP (**4**)[3] and 1,5-dimethyl-PTP (**5**)[3] were obtained from 1-methyl-4-phenylpyridinium iodide and 1,3-dimethyl-4-phenylpyridinium iodide, respectively, by reduction with sodium borohydride in methanol.[4] 1,3-Dimethyl-PTP (**7**)[3] was obtained from α-prodine (Nisentil) by hydrolysis, followed by dehydration of the alcohol with acid to produce a mixture of 1,5- and 1,3-dimethyl-PTP, which were separated by fractional crystallization of their hydrochlorides. PEPTP was obtained by N-alkylation of 4-phenyl-1,2,3,6-tetrahydropyridine (PTP) with β-phenylethyl bromide and characterized as the hydrochloride.[5]

The enzyme used in these studies was pure, catalytically active MAO B:monoclonal antibody complex (MAO B:MAO B-1C2) prepared from human autopsy liver as described by Patel et al.,[2] using an extensively characterized monoclonal antibody (MAO B-1C2), which recognizes human MAO B but not MAO A.[6,7]

In general, initial rates of reaction were determined at 30 °C from 0–5 min by measuring the increase of absorption at 340 nm for the formation of a dihydro species.[8] MAO B:MAO B-1C2 was used at a concentration of 6.3 μg per assay, and the reaction was started by addition of the substance to be tested. Assays were done in duplicate, and the data are expressed in nanomoles of product formed per minute per milligram of enzyme complex (specific activity).

For inactivation studies, MAO B:MAO B-1C2 (63 μg) was suspended in 50 mM potassium phosphate buffer, pH 7.4, and incubated at 30 °C with 1 mM MPTP, 1,3-dimethyl-PTP, or 1,5-dimethyl-PTP. At various times (0–90 min), 10-μL aliquots were removed from the sample, added to the assay cuvettes, and diluted to 1.0 mL with the assay solution which contained 3 mM benzylamine in 50 mM potassium phosphate buffer, pH 7.4. Initial rates were determined from 0–5 min at 30 °C by measuring the increase in absorption at 250 nm. The pseudo-first-order rate constant (min^{-1}) for the inactivation of MAO B:MAO B-1C2 by MPTP[8] is shown in Table I.

When PEPTP was used as substrate, special precuations were observed to avoid precipitation. The concentration of PEPTP did not exceed 0.2 mM, 25 mM sodium phosphate (pH 7.4) was substituted for potassium phosphate, and the reaction was initiated by the addition of enzyme.

Table I. Analogues of MPTP as Substrates and/or Inhibitors of Human Liver MAO B[a]

compd	sp act.,[b] nmol min^{-1} mg^{-1}	inactivation:[c] k', min^{-1}
MPTP	85.2 ± 8.0	2.64×10^{-2}
1,3-dimethyl-PTP	11.4 ± 1.8	0
1,5-dimethyl-PTP	5.3 ± 2.6	0
PEPTP	0	0

[a] Compounds listed above were tested as substrates and inactivators of pure human liver MAO B:MAO B-1C2. [b] MPTP, 1,3-dimethyl-PTP, or 1,5-dimethyl-PTP were dissolved in 50 mM potassium phosphate buffer, pH 7.4, whereas PEPTP was dissolved in 20 μL of methanol prior to dilution in water. Each substance was then assayed as a substrate of MAO B:MAO B-1C2 by incubation in phosphate buffer, pH 7.4, at 30 °C and the increase in absorption at 340 nm was measured. Concentrations of the product were calculated from the molar extinction coefficient of the corresponding dihydropyridine derivative. Final concentrations of MPTP, 1,3-dimethyl-PTP, and 1,5-dimethyl-PTP were 1 mM in 50 mM potassium phosphate and 0.2 mM PEPTP in 25 mM sodium phosphate. Initial rates of reaction were determined from 0 to 5 min. Enzymic oxidation of PEPTP was also determined by assaying hydrogen peroxide formation after 10 min at 30 °C.[12] Under identical experimental conditions, PEPTP was oxidized at <10% the rate of MPTP (5.7 and 79.8 nmol min^{-1} mg^{-1}, respectively). [c] Inactivation studies were performed by incubating 63 μg of enzyme at 30 °C with 1 mM MPTP, 1,3- or 1,5-dimethyl-PTP in 50 mM potassium phosphate buffer, or 0.4 mM PEPTP in 2 mM potassium phosphate buffer. At various time (0–90 min), 10-μL aliquots were removed, and the activity of the enzyme was assayed with benzylamine at 30 °C. For all compounds except PEPTP, the activity was assessed in 50 mM potassium phosphate, pH 7.4, and 3 mM benzylamine by measuring the increase in absorption at 250 nm from 0 to 5 min. For PEPTP, the activity of the enzyme was measured with 1 mM benzylamine, 25 mM sodium phosphate, pH 7.4, 1 mM ABTS, and 1 unit/mL of horseradish peroxidase.[9] Initial rates were determined during the first 5 min by monitoring the increase in absorption at 410 nm. All spectrophotometric assays were done with use of a Beckman DU-7U spectrophotometer.

(1) Baum, R. M. *C & E News* **1985**, *63*(36), 7.
(2) Patel, N. T.; Fritz, R. R.; Abell, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 748.
(3) All melting points were determined on a Fisher-Johns apparatus and are corrected. MPTP·HCl (**4**), mp 253–255 °C (isopropyl ether–methanol; lit.[13] mp 241–243 °C); 1,5-dimethyl-PTP·HCl (**5**·HCl), mp 187–188 °C (isopropyl ether–methanol; lit.[14] mp 189–190 °C); 1,3-dimethyl-PTP·HCl (**7**·HCl), mp 213–214 °C (isopropyl ether–ethanol; lit.[14] mp 226 °C).
(4) Gessner, W.; Brossi, A. *Synth. Commun.* **1985**, *15*, 911.
(5) PEPTP (**6**): colorless crystals, mp 70–71 °C (hexane); CIMS, m/z 264 (M^+ + 1); ^1H NMR ($CDCl_3$) δ 7.45–7.15 (m, 10 H), 6.10 (m, 1 H), 3.25 (m, 2 H), 3.50–3.00 (m, 8 H); hydrochloride salt, mp 215–218 °C dec (isopropyl ether–ethanol); anal. C, H, N, Cl.
(6) Denney, R. M.; Fritz, R. R.; Patel, N. T.; Abell, C. W. *Science* **1982**, *215*, 1400.
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Scheme I. Synthesis of the Reverse Ester of Meperidine and Its Analogues

INTERMEDIATE → Propionic anhydride, Heating → DESIRED PRODUCT + SIDE PRODUCT(S)

	R_1	R_2
1	Me	H
2	Me	Me
3	CH_2CH_2Ph	H

	R_1	R_2
4	Me	H
5	Me	Me
6	CH_2CH_2Ph	H

7

For inactivation studies, the concentration of PEPTP was 0.4 mM in 2 mM potassium phosphate buffer, pH 7.1, 10% glycerol, and 26.9 μg of MAO B:MAO B-1C2. At various times, 10-μL aliquots were removed and added to 0.5 mL of 25 mM sodium phosphate buffer, pH 7.4, 1 mM benzylamine, 1 mM 2,2′-azinobis(3-ethylbenzthiazoline 6-sulfonate) (ABTS) and 0.5 unit of peroxidase at 30 °C.[9] Initial rates were determined by monitoring the increase in absorption at 410 nm from 0 to 5 min. Benzylamine oxidation was also determined at 250 nm as described above.

In all assays, the analogues investigated were found to be inactive or much less active than MPTP (Table I). The results obtained, together with data obtained in vitro for several other analogues of MPTP[10,11] (with different *N*-alkyl groups and the phenyl substituent in the 2- or 5-position), indicate that the oxidation of MPTP by MAO B is highly structure specific in regard to the tetrahydropyridine moiety.

These studies reveal that PEPTP cannot be metabolized by MAO B in humans by a mechanism analogous to the activation of MPTP. This process seems to be necessary for neurotoxic activity of this group of compounds, but our results suggest that PEPTP probably is not toxic in vivo, unless it is activated by another mechanism.

Acknowledgment. We thank N. Patel for the preparation of MAO B, R. Cole for technical assistance, and P. Levine for helpful comments on the manuscript. Supported by the Multidisciplinary Research Program on Schizophrenia, University of Texas Medical Branch, Galveston.

Registry No. 4, 28289-54-5; 5, 13515-63-4; 6, 13314-65-3; 7, 6672-51-1; MAO, 9001-66-5.

† National Institutes of Health.
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Received October 28, 1985

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1-Ethyl-7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acid. New Quinolone Antibacterial with Potent Gram-Positive Activity

Sir:

In recent years, an old class of antibacterials known collectively as the quinolones[1] has aroused a great deal of new interest.[2] The original members of this class, oxolinic acid (**3**) and nalidixic acid (**4**), have been used for certain clinical indications for over 20 years but suffer from a rather limited antibacterial spectrum.[1] The current de-

(1) The generic class "quinolones" refers to any of the 4-pyridone-3-carboxylic acid antibacterials. These now include quinolines, naphthyridines, benzoxazines, etc., which share the common 4-pyridone-3-carboxylic acid. For a review, see: Albrect, R. *Prog. Drug Res.* **1977**, 21, 9.
(2) Many reports regarding the newer quinolones have been reported at the 23rd and the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy, Oct 24–26, 1983, Las Vegas, NV, and Oct 8–10, 1984, Washington, D.C. A portion of this work was presented at the Washington, DC, meeting; Abstr 80.

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