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PRESENCE OF TETRAHYDROISOQUINOLINE AND 2-METHYL-TETRAHYDROQUINOLINE IN PARKINSONIAN AND NORMAL HUMAN BRAINS

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1,2,3,4-Tetrahydroisoquinoline (TIQ) and 2-methyl-1,2,3,4-tetrahydroquinoline (2-Me-TQ) were identified for the first time by gas chromatography-mass spectrometry in the parkinsonian and normal human brains. TIQ, an analogue of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was markedly increased in the parkinsonian brain and could be an endogenous neurotoxin to induce Parkinson's disease.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces parkinsonian state in humans, monkeys and mice (1-3). Parkinson's disease is characterized by the degeneration of the nigrostriatal dopaminergic neurons and by the decrease of dopamine contents in the striatum (4). MPTP is a neurotoxin and causes the degeneration of nigrostriatal dopaminergic neurons (1-3). Neurotoxicity of MPTP is dependent on its conversion by monoamine oxidase type B to 1-methyl-4-phenylpyridinium ion (MPP^+) in the brain (5).

We reported that MPTP inhibited tyrosine hydroxylation in tissue slices of the striatum after a single administration and caused reduction of tyrosine hydroxylase itself after repeated administration to mice (6). We also reported that MPTP and MPP^+ inhibited tyrosine hydroxylation in tissue slices in situ, specially from the striatum, and that the inhibition by MPTP but not the effect

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of MPP^+ was prevented by a MAO inhibitor or by a dopamine uptake inhibitor (7, 8). We have developed a simple screening system to find a probable compound to induce Parkinson's disease by the inhibition of the tyrosine hydroxylase system in striatal tissue slices (9). The results of screening of various MPTP or MPP^+-related compounds indicated that both pyridinium and phenyl group are essential for the effects and that either N-methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-TIQ) or 1,2,3,4-tetrahydroisoquinoline (TIQ) that is structurally similar to MPTP and related to dopamine could be one of the candidate of endogenous or environmental factors that produce Parkinson's disease (10,11). In fact, TIQ was shown to reduce tyrosine hydroyxlase after repeated administration to mice (10,11). Kohno et al. (12) have recently reported the presence of TIQ and 1-Me-TIQ as novel endogenous amines in the rat brain by coupled gas chromatography-multiple ion detection.

We report here presence of TIQ and 2-methyl-1,2,3,4-tetrahydroquinoline (2-Me-TQ) in parkinsonian and normal human brains by gas chromatography-mass spectrometry.

MATERIALS AND METHODS

The brain was obtained at autopsy from a patient with Parkinson's disease. The patient was a 68-year-old female suffering from Parkinson's disease for 4 and half years, died suddenly from asphyxia, and the postmortem time was 5 hours. The diagnosis of Parkinson's disease was confirmed histopathologically by the neuronal loss in the substantia nigra and by the observation of the Lewy bodies. The normal control brain was from a patient with stomach cancer who died from asphyxia. The patient was a 81-year-old male, and the postmortem time was 6 hours, and no histopathological abnormalities were observed in the brain. The brains were stored at -80°C until sample preparation.

TIQ, 1,2,3,4-tetrahydroquinoline (TQ), and 2-Me-TQ were purchased from Wako Pure Chemical Industries, Ltd., heptafluorobutyric anhydride (HFBA) and pentafluoropropionic anhydride (PFPA) from Gasukuro Kogyo Inc. All other chemicals used were of analytical grade.

The brain samples were treated for gas chromatography-mass spectrometry, as described by Kohno et al. (12). The frontal cortex (25 g) was homogenized for 30 sec at 0°C in 0.4 N perchloric acid (20 ml) containing EDTA (0.1% w/v) and ascorbic acid (0.1% w/v). The homogenate was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was transferred to a glass test tube, and the pellet was vortexed with 0.4 N perchloric acid (20 ml) containing EDTA (0.1% w/v) and ascorbic acid (0.1% w/v) and centrifuged again. The combined supernatant was extracted with diethyl ether (20 ml). The aqueous phase was adjusted to pH 11.0 with 6 N NaOH and extracted twice with dichloromethane (20 ml). The organic phase was extracted with 0.1 N HCl solution (20 ml) containing EDTA (0.1% w/v) and ascorbic acid (0.1% w/v). The aqueous phase was adjusted to pH 11.0 and extracted with dichloromethane (20 ml). The organic phase was dehydrated over anhydrous sodium sulfate and the filtrate evaporated to dryness with N_2 stream. The residue was dissolved in ethyl acetate/HFBA (20 µl: 20 µl) or ethyl acetate/PFPA (20 µl: 20 µl), and derivatized at 70°C for 30 min. For identifica-

tion no chemical was added as an internal standard, but for semiquantitation a constant amount of TQ was added to the supernatant of the homogenate as an internal standard.

For gas chromatography-mass spectrometry (GC/MS), a Shimadzu gas chromatograph (GC-9A)-mass spectrometer (9020-DF) was used. Gas chromatography was performed on a HiCap-CBP1-M25-025 fused-silica capillary column (25 m x 0.2 mm I.D.), film thickness 0.25 μm (Shimadzu), with helium (23 cm/sec) as a carrier gas. The samples were injected solventless with a moving-needle type injector. The injection temperature was 250°C, temperature program from 130 to 190°C at 3°C/min; separator temperature 280°C, ion source temperature 250°C, electron-impact ionization (EI) energy 70 eV, trap current 60 μA, and accelerating voltage 3 kV. Chemical ionization (CI) mass spectrometry was performed with isobutane as a reactant gas. CI energy was 200 eV and emission current 200 μA. The other conditions were the same as for EI.

RESULTS

Fig. 1 shows mass chromatograms of the HFB derivatives of 2-Me-TQ and TIQ (a), and the HFB derivatized extracts from frontal cortex of a patient with Parkinson's disease (b) and from a control patient (c).

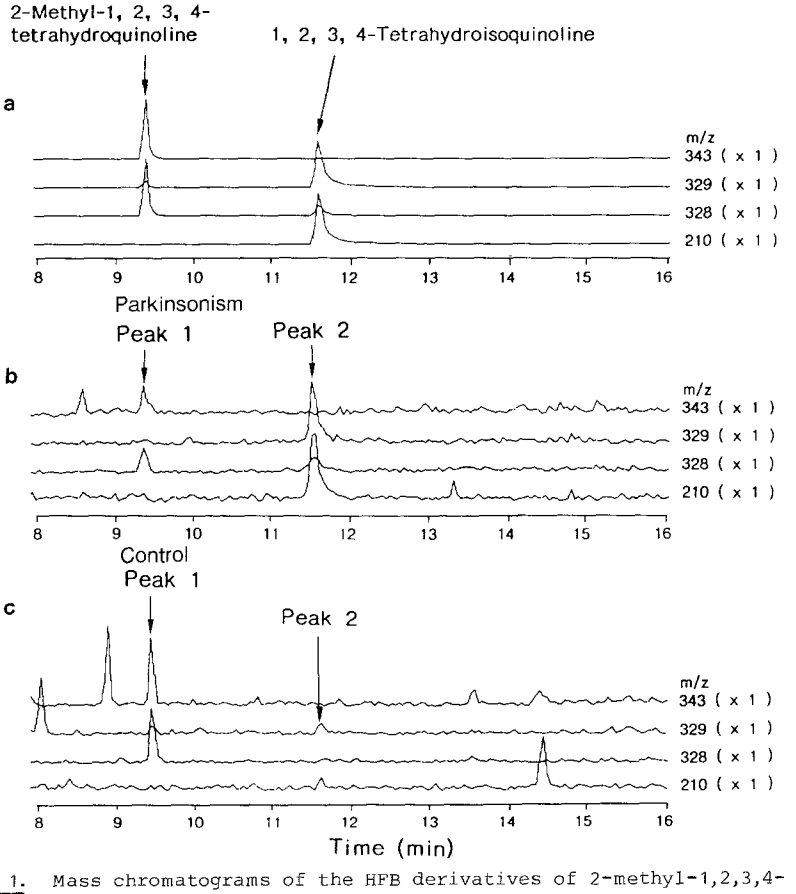

Figure 1. Mass chromatograms of the HFB derivatives of 2-methyl-1,2,3,4-tetrahydroquinoline (2-Me-TQ) and 1,2,3,4-tetrahydroisoquinoline (TIQ) (a), the HFB derivatized extracts from parkinsonian frontal cortex (b) and normal frontal cortex (c).

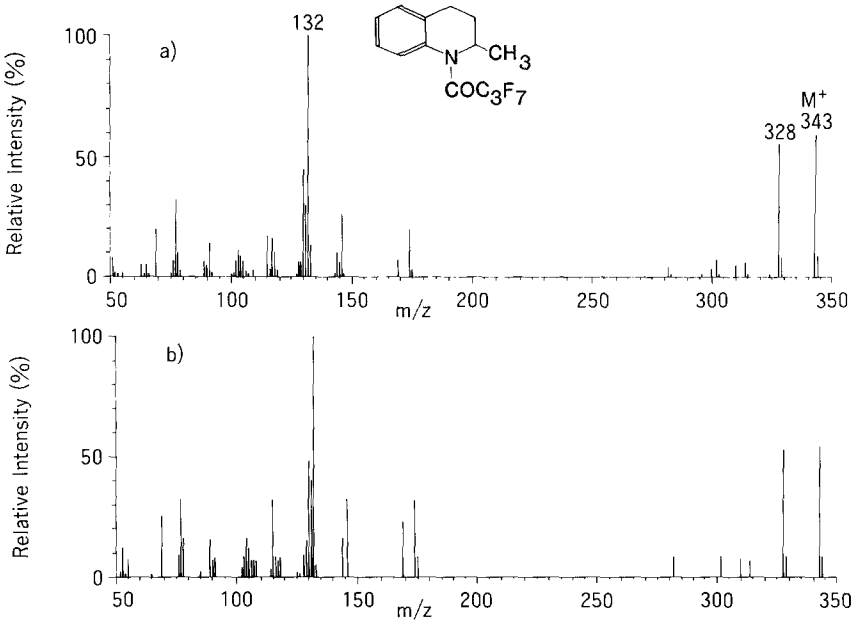

Figure 2. EI mass spectra of the HFB derivative of 2-Me-TQ (a), and peak 1 (b) in Fig. 1.

EI mass spectrum of peak 1 in Fig. 1 (b) is shown in Fig. 2 (b). CI mass spectrum of peak 1 revealed an intense quasimolecular ion at m/z 344, $[M+H]^+$, indicating the molecular ion at m/z 343. Peak 1 was identified as 2-Me-TQ since it showed identical retention time on GC (Fig. 1) and almost identical EI mass spectrum (Fig. 2) to those of the HFB derivative of the authentic compound. The same result was obtained with peak 1 in Fig. 1 (c).

EI mass spectrum of peak 2 in Fig. 1 (b) is shown in Fig. 3 (b). CI mass spectrum of peak 2 also showed an intense quasimolecular ion at m/z 330, $[M+H]^+$, indicating the molecular ion at m/z 329. Peak 2 was identified as TIQ since it showed identical retention time on GC (Fig. 1) and almost identical EI mass spectrum (Fig. 3) to those of the HFB derivative of the authentic compound.

The detection of 2-Me-TQ in the frontal cortex of a patient with Parkinson's disease and of a control patient was confirmed by using PFP derivatization instead of HFB. The detection of TIQ was also confirmed by using PFP derivatization in the frontal cortex of the parkinsonian patient, but could not be confirmed in the frontal cortex of the control patient by using PFP derivatiza-

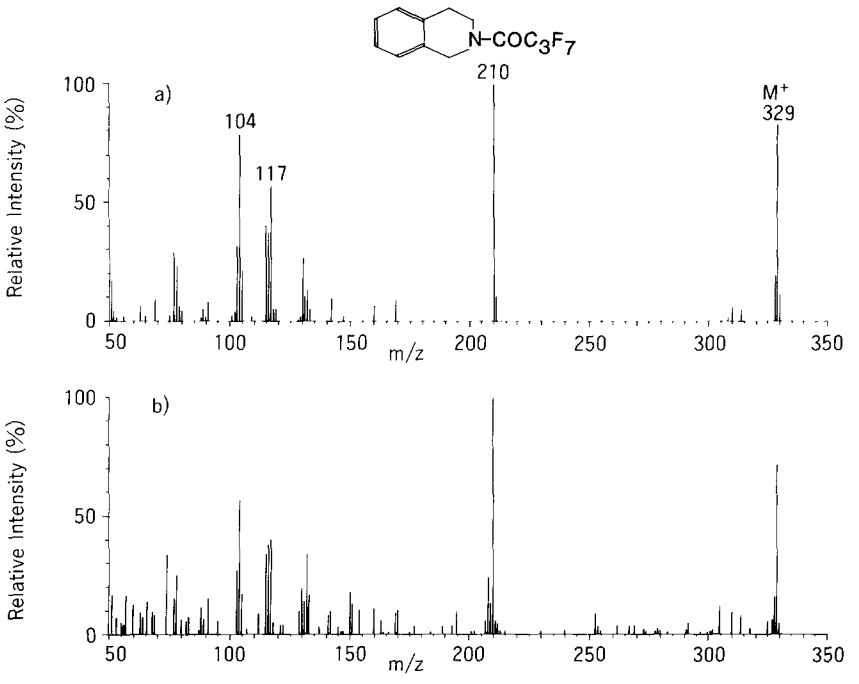

Figure 3. EI mass spectra of the HFB derivative of TIQ (a), and peak 2 (b) in Fig. 1.

tion, probably due to the lower concentration of TIQ in the control patient, as compared with that of the parkinsonian patient.

By selective ion monitoring, the concentration of TIQ in the frontal cortex of the parkinsonian patient was found to be markedly increased as compared with that of the control patient; approximately 10 ng/g in the parkinsonian brain and less than 1 ng/g in the control brain. In contrast, the concentration of 2-Me-TQ was similar between the parkinsonian brain (2 ng/g) and the normal brain (3 ng/g).

DISCUSSION

The discovery of MPTP, which is a neurotoxin specific for the nigrostriatal dopaminergic neurons and produces Parkinson's disease (1-3), suggests that Parkinson's disease might be caused by some endogenous or exogenous neurotoxins similar to MPTP. We have screened the compounds structurally similar to MPTP or MPP^+ based on the inhibition of tyrosine hydroxylation in striatal tissue slices (7-9) and have reported that TIQ or N-Me-TIQ could be a candidate for the endogenous neurotoxins (10,11). In fact, TIQ was found to reduce tyrosine

hydroxylase in the striatum after repeated peripheral administration to mice (10,11). Kohno et al. (12) have recently found TIQ and 1-Me-TIQ in the rat brain. 6,7-Dihyroxy-1-Me-TIQ was also found in the rat brain (13).

In the present study, we have confirmed for the first time the presence of TIQ and 2-Me-TQ in the brains from a parkinsonian patient and a control patient. 2-Me-TQ is a novel amine that has never been identified in animals. The level of TIQ was much higher in the parkinsonian brain than that in the normal brain, whereas the level of 2-Me-TQ was similar between the parkinsonian brain and the normal brain (Fig. 1). We could not detect in the parkinsonian or normal brain 1-Me-TIQ that was found in the rat brain by Kohno et al. (12). Although further studies are required, the present results suggest the possibility that TIQ which is structurally related to MPTP and dopamine and has an *in vivo* effect similar to that of MPTP (10,11) could be a candidate for the endogenous neurotoxin to produce Parkinson's disease.

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