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Short communication

Detection of 1-phenyl-N-methyl-1,2,3,4-tetrahydroisoquinoline and 1-phenyl-1,2,3,4-tetrahydroisoquinoline in human brain by gas chromatography–tandem mass spectrometry

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Abstract

1-Phenyl-N-methyl-1,2,3,4-tetrahydroisoquinoline and 1-phenyl-1,2,3,4-tetrahydroisoquinoline were detected for the first time in parkinsonian human brain using gas chromatography–tandem mass spectrometry (GC–MS–MS). Since these compounds are structural analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that produces parkinsonism in humans, they might be candidates for endogenous MPTP-like neurotoxins.

1. Introduction

Since the discovery of a highly selective, irreversible neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that produces parkinsonism in humans, monkeys, and mice [1-3], endogenous and exogenous MPTP-like compounds that accumulate in the brain and induce parkinsonism were extensively searched. 1,2,3,4-Tetrahydroisoquinoline (TIQ) was detected in the human brain [4-6], and subcutaneous injection of TIQ produced parkinsonism in primates with a decrease in the amounts of dopamine and biopterin, a reduction of the activity of tyrosine hydroxylase in the nigrostriatal regions [7,8] and with accumulation of TIQ in the brains [9]. Death of the dopamine neurons was not observed even after chronic administration of TIQ for up to 104 days in monkeys [8]. TIQ is methylated to form N-methyl-1,2,3,4-tetrahydroisoquinoline (NMTIQ) [10,11]. NMTIQ is oxidized by monoamine oxidase in human brain to N-methylisoquinolinium ion [12] which is more neurotoxic. Since TIQ was widely detected in various foods [13,14], it was speculated that TIQ from foods may accumulate in the

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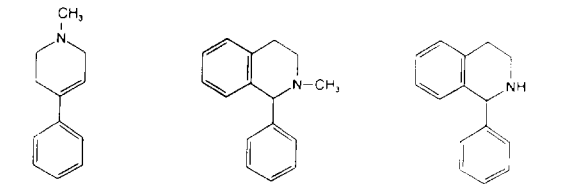

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) | 1-Phenyl-N-methyl-1,2,3,4-tetrahydroisoquinoline (Phenyl-NMTIQ) | 1-Phenyl-1,2,3,4-tetrahydroisoquinoline (Phenyl-TIQ)

Fig. 1. Chemical structures of MPTP, phenyl-NMTIQ and phenyl-TIQ.

human brain over a long period, causing Parkinson's disease. However, the concentration of TIQ was not elevated in the brains of patients with Parkinson's disease as compared to control brains.

Thus, an extensive search for other endogenous MPTP-like compounds has been undertaken. 1-Phenyl-N-methyl-1,2,3,4-tetrahydroisoquinoline (phenyl-NMTIQ) and 1-phenyl-1,2,3,4-tetrahydroisoquinoline (phenyl-TIQ) were postulated to be produced endogenously from phenylethylamine. Fig. 1 shows the chemical structure of MPTP, phenyl-NMTIQ and phenyl-TIQ. Phenyl-NMTIQ and phenyl-TIQ are structurally similar to MPTP. This study first demonstrates the presence of phenyl-NMTIQ and phenyl-TIQ in human brain as possible endogenous MPTP-like compounds.

2. Experimental

2.1. Materials

Human brains were obtained from a 61-year-old male control patient with liver cirrhosis and renal failure, a 74-year-old male with Parkinson's disease and a 80-year-old female with Parkinson's disease, 3 h, 4 h and 8 h, respectively, after death. Phenyl-TIQ and phenyl-NMTIQ were synthesized according to the method of Gray et al. [15]. The synthesized compounds were confirmed to be pure by NMR spectroscopy and mass spectrometry.

2.2. Sample preparation

The frontal cortex of brain (5 g) was homogenized with 0.4 *M* perchloric acid (5 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 *M* perchloric acid (5 ml) containing EDTA (0.1%, w/v) and ascorbic acid (0.1%, w/v), and the mixture was centrifuged again. The combined supernatant was extracted with diethyl ether (10 ml). The aqueous phase was adjusted to pH 11 with 6 *M* NaOH and extracted twice with dichloromethane (10 ml). The organic phase was extracted with 0.1 *M* HCl (10 ml) containing EDTA (0.1%, w/v) and ascorbic acid (0.1%, w/v). The aqueous phase was adjusted to pH 11 with 6 *M* NaOH and extracted with dichloromethane (10 ml). The organic phase was dehydrated over anhydrous sodium sulphate, and the filtrate was evaporated to dryness under a stream of nitrogen. The residue was dissolved with 10 μl of ethyl acetate, and 2 μl of sample was analyzed with gas chromatography–tandem mass spectrometry (GC–MS–MS).

2.3. GC–MS–MS

A tandem mass spectrometer, TSQ-700 (Finnigan MAT, San Jose, CA, USA), was used. The gas chromatograph was equipped with a splitless injector and a DB-17 capillary column (20 m × 0.25 mm I.D., film thickness 0.25 μm). The conditions of GC–MS–MS were: the injection temperature, 250°C; the column temperature program, from 180°C for 1 min to 230°C at 5°C/min; the transfer line temperature, 280°C; the chemical ionization (CI) gas, methane; the filament current, 200 μA; the collision cell, −25 kV; the collision gas, argon. To detect phenyl-TIQ and phenyl-NMTIQ in human brain, selected reaction monitoring (SRM) was used. Even though there was no internal standard to correct for extraction inefficiencies, a rough estimate of phenyl-TIQ and phenyl-NMTIQ in the brain was calculated using the peak heights of the product ions at *m/z* 105 as compared with

those of a known amount of standards. The detection limits of the compounds using SRM were approximately 5 pg.

3. Results

Fig. 2 shows the CI-MS spectrum of authentic phenyl-NMTIQ (a) and the collision-induced dissociation (CID) mass spectrum of the m/z 224 ion, $(M + H)^+$, of phenyl-NMTIQ (b). The CID spectrum of the precursor ion at m/z 224 showed product ions at m/z 91, 105, 178 and 193, which were used for the SRM method of GC–CI-MS–MS to detect phenyl-NMTIQ in human brains

Fig. 3 shows the SRM chromatograms of authentic phenyl-NMTIQ (a), and the extracts from parkinsonian brain (b) and control brain

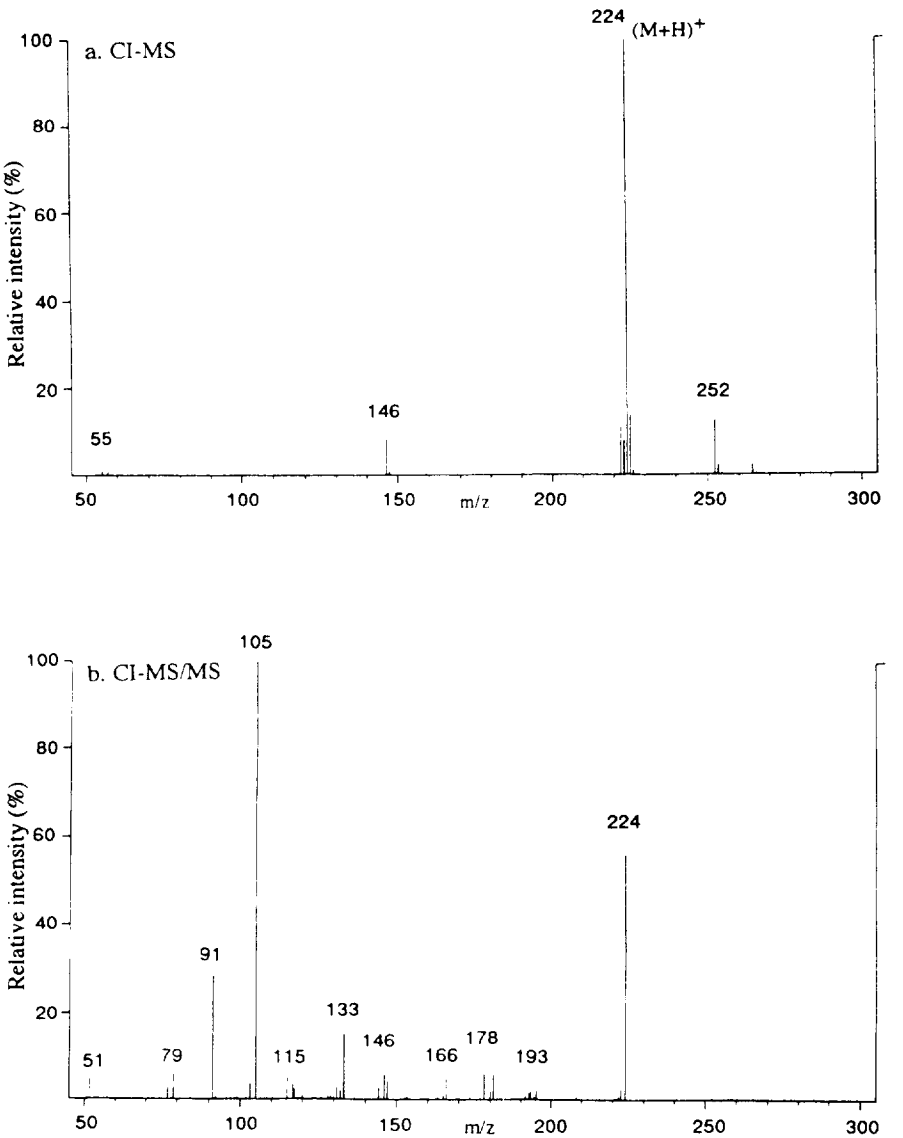

Fig. 2. CI–MS spectrum of authentic phenyl-NMTIQ (a) and CID mass spectrum of the m/z 224 ion, $(M + H)^+$, of phenyl-NMTIQ (b).

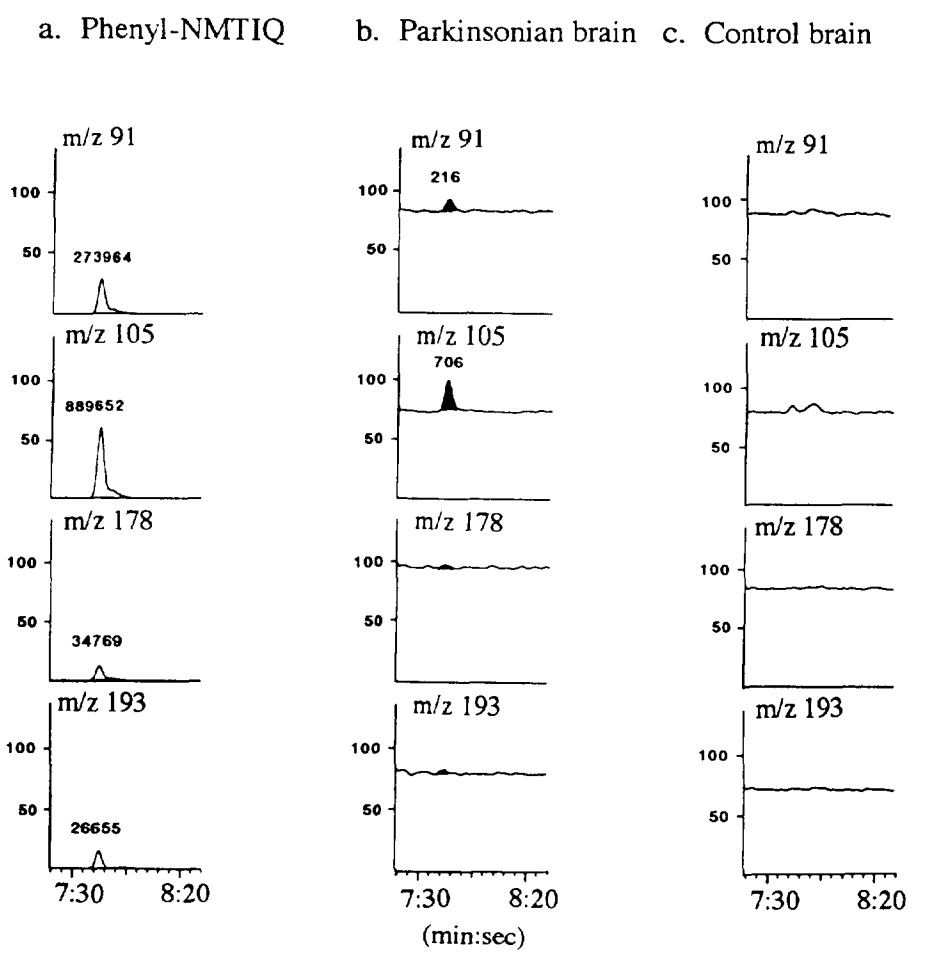

Fig. 3. SRM chromatograms of authentic phenyl-NMTIQ (a), and the extracts from parkinsonian brain (b) and control brain (c). GC–CI-MS–MS, precursor ion: *m/z* 224.

(c). Phenyl-NMTIQ was eluted at 7:43 (min:s). The SRM chromatograms demonstrated that phenyl-NMTIQ was present in parkinsonian brain. The peaks in parkinsonian brain showed identical retention times on the chromatograms and almost identical peak height ratios such as *m/z* 91 to *m/z* 105, to those of the authentic phenyl-NMTIQ.

Fig. 4 shows the CI-MS spectrum of authentic phenyl-TIQ (a) and the CID mass spectrum of the *m/z* 210 ion, $(M + H)^+$, of phenyl-TIQ (b). The CID spectrum of the precursor ion at *m/z* 210 showed product ions at *m/z* 91, 105, 178 and 193, which were used for the SRM method of GC–MS–MS to detected phenyl-TIQ in the human brain.

Fig. 5 shows the SRM chromatograms of authentic phenyl-TIQ (a), and the extracts from parkinsonian brain (b) and control brain (c). Phenyl-TIQ was eluted at 9:20 (min:s). The SRM chromatograms demonstrated that phenyl-TIQ was present in the human brain, since the peaks in the brain showed identical retention times on the chromatograms and almost identical peak height ratios such as *m/z* 91:*m/z* 105, and *m/z* 178:*m/z* 105, as for the authentic phenyl-TIQ.

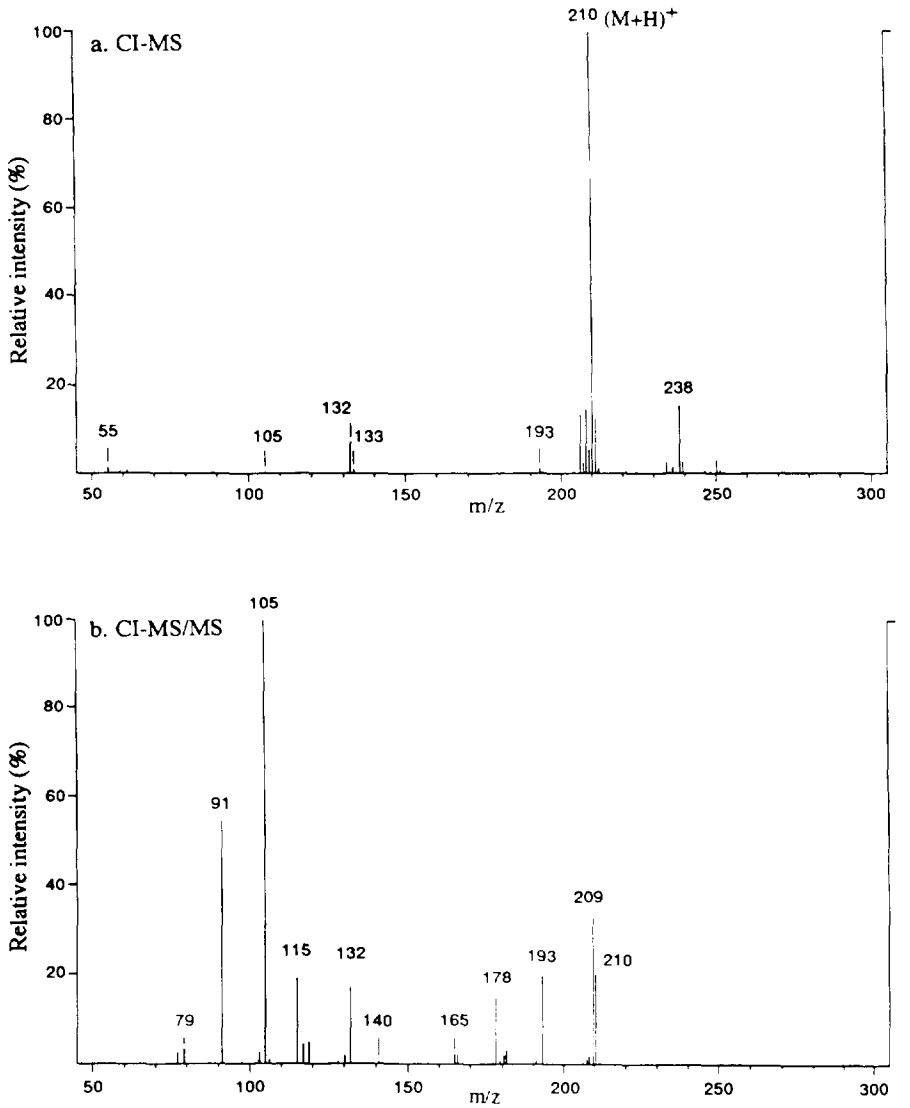

Fig. 4. CI–MS spectrum of authentic phenyl-TIQ (a) and CID mass spectrum of the m/z 210 ion, $(M + H)^+$, of phenyl-TIQ (b).

Thus, phenyl-NMTIQ was detected only in the brains from two patients with Parkinson's disease (approximately 14 and 43 pg/g wet tissue) but not in the brain from a control patient. However, phenyl-TIQ was detected in the brains from the patients with Parkinson's disease (approximately 34 and 12 pg/g wet tissue) and a control patient (approximately 19 pg/g wet tissue).

4. Discussion

Phenyl-NMTIQ and phenyl-TIQ could be detected in human brain. Since these compounds, especially phenyl-NMTIQ, are structural analogues of MPTP, they might be candidates for endogenous MPTP-like neurotoxins.

We used the SRM method of GC–MS–MS to detect trace phenyl-NMTIQ and phenyl-TIQ in

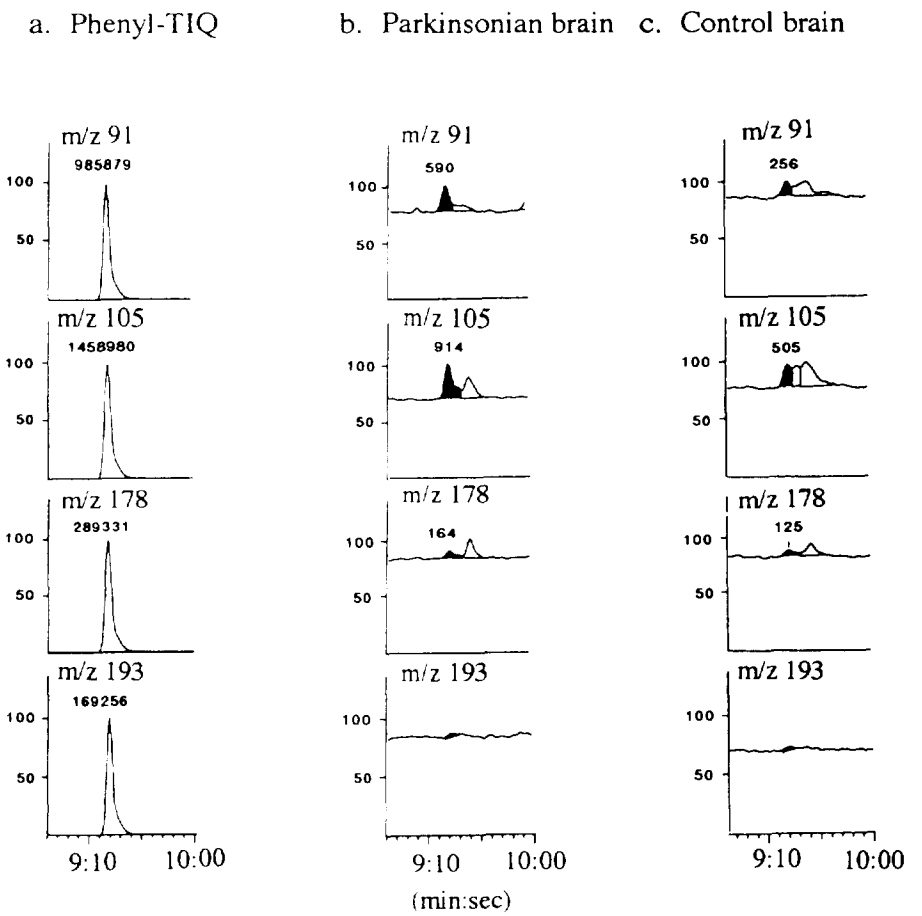

Fig. 5. SRM chromatograms of authentic phenyl-TIQ (a), and the extracts from parkinsonian brain (b) and control brain (c). GC–CI-MS–MS, precursor ion: *m/z* 210.

the brain. SRM monitors selected product ions produced by CID of a particular precursor ion. In a triple quadrupole instrument for SRM, the first quadrupole is set to pass the precursor ion and the third quadrupole is set to pass the appropriate product ions. The second quadrupole acts as a collision cell. SRM can provide a greater selectivity and sensitivity as compared to the selected ion monitoring method of GC–MS.

Phenyl-TIQ may be nonenzymatically synthesized by in vivo Pictet–Spengler condensation of phenylethylamine and benzaldehyde that has been detected in the serum and urine of healthy subjects [16–18]. However, the physiological relevance of benzaldehyde in the brain is not yet known, especially in the brain, and the production of phenyl-TIQ from phenylethylamine and benzaldehyde is only one of the possible pathways for endogenous synthesis of phenyl-TIQ. The presence of phenyl-NMTIQ in the brain indicates that phenyl-NMTIQ and phenyl-TIQ, a precursor of phenyl-NMTIQ, are not artifacts during sample preparation, because phenyl-NMTIQ cannot be produced nonenzymatically from benzaldehyde and phenylethylamine, but can be enzymatically produced from N-methylation of phenyl-TIQ by N-methyltransferase. We demonstrated that N-methylation of TIQ into NMTIQ occurred in vitro by N-methyltransferase in the human brain

[10] and in vivo in the brain of a TIQ-injected monkey [11], and that N-methylation of salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) into N-methylsalsolinol (1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) occurred in rat brain during in vivo microdialysis with salsolinol [19,20].

MPTP itself does not show direct neurotoxicity, but its oxidized metabolite, 1-methyl-4-phenylpyridine ion shows direct neurotoxicity to nigrostriatal dopaminergic neurons [21]. Since NMTIQ was oxidized to N-methyl-isoquinolinium ion by monoamine oxidase in human brain [12], phenyl-NMTIQ may also be oxidized by the enzyme to 1-phenyl-N-methyl-isoquinolinium ion that is expected to be more neurotoxic. The neurotoxicity of phenyl-NMTIQ and phenyl-TIQ is under investigation by our group to determine if the compounds are involved in the pathogenesis of Parkinson's disease.

Acknowledgements

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