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Note

Detection of tetrahydroisoquinoline in parkinsonian brain as an endogenous amine by use of gas chromatography–mass spectrometry

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Since the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces parkinsonism in humans, monkeys and mice [1–3], the hypothesis that an endogenous or environmental neurotoxin structurally similar to MPTP causes Parkinson's disease has been strongly proposed. Although MPTP itself does not show direct neurotoxicity, MPTP passes easily

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through the blood–brain barrier, is oxidized to the 1-methyl-4-phenyl-2,3-dihydropyridinium ion ($MPDP^+$) by monoamine oxidase type B in glial cells and is then further oxidized enzymatically or non-enzymatically to the 1-methyl-4-phenylpyridinium ion (MPP^+), which shows direct neurotoxicity to nigrostriatal dopaminergic neurons [4].

Hirata and Nagatsu [5] demonstrated that a single administration of MPTP inhibited the activity of tyrosine hydroxylase in tissue slices of the striatum, and that repeated administration of MPTP reduced tyrosine hydroxylase itself. Further, they showed [6,7] that MPTP and MPP^+ inhibited tyrosine hydroxylase in tissue slices of striatum in situ. After screening the possible neurotoxic compounds structurally similar to MPTP by assay of inhibition of tyrosine hydroxylase in striatal tissue slices, we found that the pyridinium and phenyl rings were crucial for the effects, and N-methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-TIQ) and 1,2,3,4-tetrahydroisoquinoline (TIQ) were suggested as endogenous MPTP-like compounds [8–10]. Repeated administration of TIQ to mice caused reduction of tyrosine hydroxylase [9,10], and in monkeys it produced parkinsonian symptoms with reduction in dopamine and tyrosine hydroxylase [11].

TIQ was first identified in rat brain by Kohno et al. [12]. We detected TIQ for the first time in the parkinsonian and normal human brains [13]. The presence of TIQ in human brains was also reported by Ohta et al. [14]. In these studies [12–14], TIQ was measured by gas chromatography–mass spectrometry (GC–MS) after its extraction from the brain tissue and the derivatization reaction with heptafluorobutyric anhydride (HFBA). However, there is a question about the endogenous origin of TIQ in the brain. TIQ could be formed as an analytical artifact by the reaction of endogenous 2-phenylethylamine with formaldehyde, a possible contaminant in organic solvents. It is also possible that some phenylethylamine present as N-methylenephenylethylamine in brain tissue is artifactually cyclized to form TIQ by HFBA or heptafluorobutyric acid (Dr. A.A. Boulton, personal communication). We demonstrate in this study that TIQ was not an artifact but an endogenous compound in the brain; we used either [$^2H_4$]phenylethylamine added to the homogenized tissue or a gentle derivatization method with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA).

EXPERIMENTAL

Chemicals

TIQ was purchased from Wako Pure Chemical Industries (Osaka, Japan), HFBA from Gasukuro Kogyo (Osaka, Japan), BSTFA from Pierce (Rockford, IL, U.S.A.) and 2-phenylethyl[1,1,2,2-$^2H_4$]amine (98.8 atom %) from MSD Isotopes (Montreal, Canada). All other chemicals used were of analytical grade.

Samples

The brain was obtained from a 68-year-old, female patient with Parkinson's disease. She had been suffering from Parkinson's disease for 4.5 years, until she died suddenly from asphyxia. The diagnosis of Parkinson's disease was confirmed by histopathological observation of nigrostriatal neuronal loss and Lewy's body. The brain was stored at −70°C until sample preparation.

Sample preparation

To demonstrate that TIQ is not an analytical artifact but an endogenous compound, the parkinsonian brain was homogenized after addition of 2-phenylethyl[1,1,2,2-$^2H_4$]amine and then prepared for GC–MS as described in the previous literature [13] with some modifications. The occipital cortex (20 g) spiked with 20 or 200 μg of 2-phenylethyl[1,1,2,2$^2H_4$]amine was homogenized with 0.4 *M* 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 *M* perchloric acid (20 ml) containing EDTA (0.1%) and ascorbic acid (0.1%) and centrifuged again. The combined supernatant was extracted with diethyl ether (20 ml). The aqueous layer was adjusted to pH 11.0 with 6 *M* sodium hydroxide and extracted twice with dichloromethane (20 ml). The organic phase was dehydrated over anhydrous sodium sulphate, and the filtrate was evaporated to dryness under a nitrogen stream. The residue was dissolved in 40 μl of ethyl acetate–HFBA (1:1) and derivatized at 70°C for 30 min.

To demonstrate that TIQ is not an artifact during HFB derivatization from some phenylethylamine present as N-methylenephenylethylamine in brain tissue but an endogenous compound, the extract from the parkinsonian brain (20 g) was gently trimethylsilylated overnight with 20 μl of BSTFA at room temperature. The extraction was performed as described above, but without addition of deuterated phenylethylamine.

Gas chromatography–mass spectrometry

We used a Shimadzu GC-9A gas chromatograph combined with a double-focusing mass spectrometer (Shimadzu 9020-DF). The chromatograph was equipped with an OV-1 bonded fused-silica capillary column (25 m×0.25 mm I.D.) and a moving-needle-type solventless injector. The injection temperature was 280°C and the column temperature was programmed from 130°C to 190°C at 3°C/min. Electron-impact ionization (EI) mass spectra were recorded at an ionizing energy of 70 eV, an ion source temperature of 250°C, a trap current of 60 μA and an accelerating voltage of 3 kV.

RESULTS

Fig. 1 shows gas chromatograms and mass chromatograms of the HFB derivative of TIQ (A) and the HFB-derivatized extract from the occipital cortex of a patient with Parkinson's disease, spiked with 20 μg of 2-phenylethyl[1,1,2,2-$^2H_4$]amine just before homogenization (B). The EI mass spectrum of the HFB derivative of authentic TIQ is shown in Fig. 2A. The EI mass spectrum of peak 1 in Fig. 1B is shown in Fig. 2B. Peak 1 in Fig. 1B was identified as TIQ, since it showed almost an identical EI mass spectrum and an identical retention time on the mass chromatogram with those of HFB deriv-

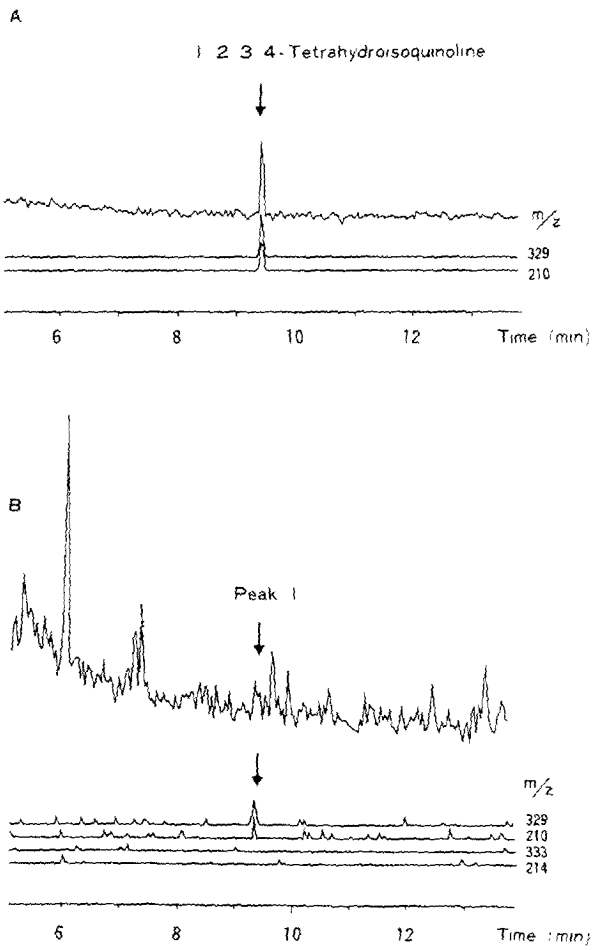

Fig. 1. Gas chromatograms and mass chromatograms of the HFB derivative of authentic TIQ (A) and the HFB-derivatized extract from the occipital cortex of a patient with the Parkinson's disease, spiked with 20 μg of 2-phenylethyl[1,1,2,2-$^2H_4$]amine just before homogenization (B).

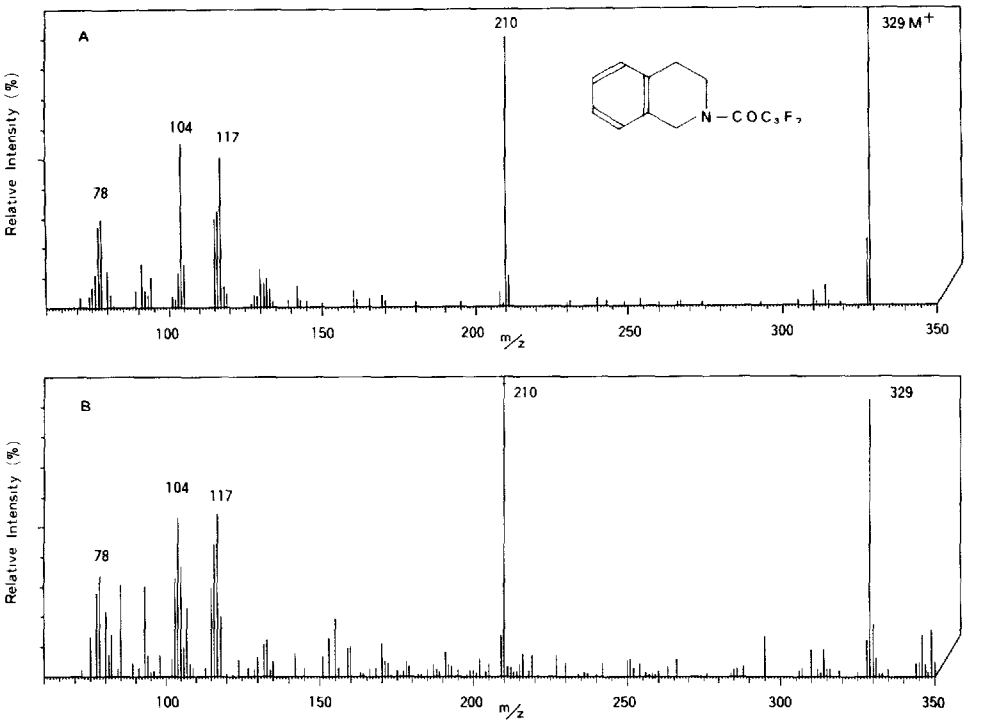

Fig. 2. (A) Mass spectrum of the HFB derivative of authentic TIQ. (B) Mass spectrum of the endogenous amine shown as peak 1 in Fig. 1B.

ative of TIQ. However, no [$^2H_4$]TIQ was detected in the brain extract spiked with 20 μg of 2-phenylethyl[1,1,2,2-$^2H_4$]amine, since no peaks were observed at *m/z* 333 (molecular ion of [$^2H_4$]TIQ) and *m/z* 214 (intense fragment ion of [$^2H_4$]TIQ) on the mass chromatogram with a retention time of a few seconds earlier than TIQ. Further, [$^2H_4$]TIQ was not detected in the brain extract spiked with 200 μg of 2-phenylethyl[1,1,2,2-$^2H_4$]amine. The concentration of TIQ in the patient's brain was determined by selected-ion monitoring (SIM), using synthesized [1,3,4-$^2H_3$]TIQ as an internal standard, to be ca. 7 ng/g wet tissue.

Fig. 3 shows the mass spectrum of the trimethylsilylated authentic TIQ. To detect the trimethylsilylated TIQ in an extract from the parkinsonian brain, SIM at *m/z* 205 (M^+), *m/z* 204 and *m/z* 190 was performed. Fig. 4 shows the SIM chromatograms of the trimethylsilylated authentic TIQ (A) and the trimethylsilylated extract from the parkinsonian cortex (B). The presence of trimethylsilylated TIQ in the extract from the parkinsonian brain was confirmed by the retention time, the characteristic selected mass numbers and also by the ion intensity ratio for TIQ (*m/z* 205, 204, 190).

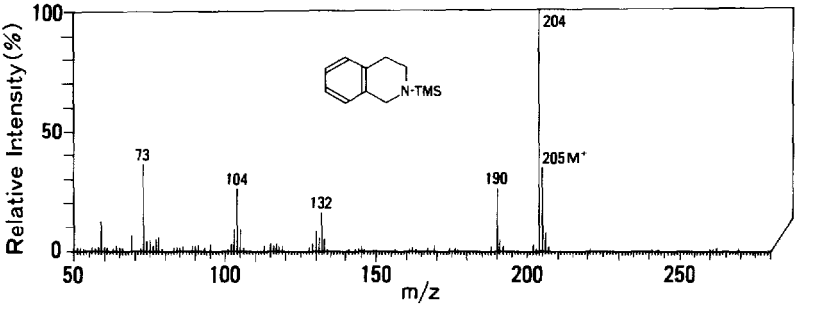

Fig. 3. Mass spectrum of trimethylsilylated authentic TIQ.

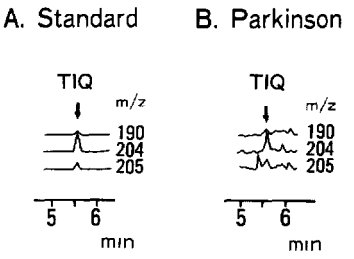

Fig. 4. (A) SIM chromatogram of trimethylsilylated authentic TIQ. (B) SIM chromatogram of the trimethylsilylated extract from the parkinsonian cortex.

DISCUSSION

TIQ was detected in parkinsonian brain as an endogenous amine and was demonstrated not to be an artifact produced during sample work-up and HFB derivatization. Since formaldehyde often contaminates many organic solvents, it may react with endogenous 2-phenylethylamine, thus giving rise to artifactual formation of TIQ. Therefore, the use of deuterium-labelled 2-phenylethylamine was the only reliable way of demonstrating by means of GC–MS that TIQ was not an artifact but was an endogenous amine in the brain. Since the artifactual formation of TIQ could also occur from endogenous N-methylenephenylethylamine by cyclization during HFB derivatization, a completely different very gentle derivatization, i.e. trimethylsilylation at room temperature, was used to demonstrate that TIQ was truly present in the parkinsonian brain.

TIQ has not been found to be non-enzymically formed from 2-phenylethylamine and formaldehyde under physiological conditions [15]. A similar mechanism, which is known as the Pictet–Spengler isoquinoline synthesis [16], is suggested for the formation of tetrahydroisoquinoline derivatives, such as salsolinol [17]. The Pictet–Spengler reaction is formation of tetrahydroisoquinoline derivatives by condensation of β-arylethylamine with carbonyl compounds and cyclization of the Schiff base formed. This condensation is

facilitated by electron-donating substituents, such as hydroxy or alkoxy groups, on the aryl groups [15]. Dopamine, which has hydroxy groups on the phenyl ring, can easily condense with acetaldehyde derived from alcohol to form salsolinol. Lauwers et al. [18] showed that formaldehyde is enzymically formed from 5-methyltetrahydrofolic acid with enzymes prepared from pig brain or rat kidney. Therefore, a possible mechanism for the synthesis of TIQ in human brain is enzymic formation from 2-phenylethylamine and formaldehyde, a part of which, at least, is enzymically formed from 5-methyltetrahydrofolic acid. However, no enzymic methods for formation of TIQ have been reported. Another possibility is that TIQ in the brain may be derived from foods, since TIQ is present in various foods [19,20] and easily passes through the blood–brain barrier [21].

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