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Note

Presence of tetrahydroisoquinoline, a parkinsonism-related compound, in foods

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Since a selective, irreversible neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that causes parkinsonism in humans, monkeys and mice [1–4] was discovered, an endogenous or environmental neurotoxin that induces Parkinson's disease has been extensively sought.

Hirata and Nagatsu [5–7] screened various compounds structurally similar to MPTP for neurotoxicity by assaying the inhibition of tyrosine hydroxylase (EC 1.14.16.2) activity in tissue slices of striatum in situ. The results of the screening indicated that both pyridinium and a phenyl ring were essential for the effects and that N-methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-TIQ) and 1,2,3,4-tetrahydroisoquinoline (TIQ) could be one of the candidates of endogenous or environmental factors that cause Parkinson's disease [8–10].

TIQ has recently been discovered in rat brain [11] and in parkinsonian and normal human brains [12,13]. Repeated administration of TIQ to marmosets

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caused the accumulation of TIQ in the brains [14] and induced a parkinsonian state with reduction of tyrosine hydroxylase, dopamine and total biopterin concentrations in the substantia nigra [15]. TIQ has also been detected in cheese, wine and cocoa [16]. The origin of TIQ in human brains could be derived from foods. We demonstrated in this study that TIQ was commonly present in various foods.

EXPERIMENTAL

Chemicals

TIQ was purchased from Wako (Osaka, Japan), heptafluorobutyric anhydride (HFBA) from Gaskuro Kogyo (Tokyo, Japan), and 1,3,4,5,6,7,8-heptadeuteroisoquinoline ([$^2H_7$]isoquinoline) from MSD Isotopes (Montreal, Canada).

A mixture of 1,3,5,6,7,8-hexadeutero-1,2,3,4-tetrahydroisoquinoline ([$^2H_6$]TIQ) and 1,3,4,5,6,7,8-heptadeutero-1,2,3,4-tetrahydroisoquinoline ([$^2H_7$]TIQ) was synthesized by a modified method of Wedekind and Oechslen [17]. [$^2H_7$]Isoquinoline was dissolved in 37% hydrochloric acid and stirred with tin powder at 60°C for 8 h, until the absorption at 325 nm had disappeared. The excess of tin was removed by filtration and the filtrate was evaporated to dryness. Water was added to the residue and the mixture was made alkaline with sodium hydroxide and extracted with chloroform. The chloroform solution was then extracted with 2 *M* hydrochloric acid and the extract was evaporated to dryness. After treatment with ethanol, the residue gave the product as small prisms. The synthesized compound was analysed by gas chromatography–mass spectrometry (GC–MS) and found to be a mixture of [$^2H_6$]TIQ and [$^2H_7$]TIQ (1:1). The structures of [$^2H_6$]TIQ and [$^2H_7$]TIQ were assigned by ^1H NMR spectroscopy.

All other chemicals were of analytical-reagent grade.

Sample preparation

To determine the TIQ levels in cheese (5 g), banana (20 g), broiled sardine (20 g), broiled beef (20 g), flour (20 g), the yolk of boiled egg (20 g) and the white of boiled egg (20 g), the food was homogenized for 30 s at 0°C in 5 volumes of 0.4 *M* perchloric acid containing EDTA (0.1%, w/v) and ascorbic acid (0.1% w/v). To the homogenate was added 50 ng of a mixture of [$^2H_6$]TIQ and [$^2H_7$]TIQ as an internal standard and the mixture was centrifuged at 10 000 *g* for 20 min at 4°C. The supernatant was transferred to a glass test-tube, adjusted to pH 12 with 6 *M* sodium hydroxide solution and extracted with 5 volumes of dichloromethane. The organic phase was extracted with 5 volumes of 0.1 *M* hydrochloric acid containing EDTA (0.1%, w/v) and ascorbic acid (0.1%, w/v). The aqueous phase was adjusted to pH 12 with 6 *M* sodium hydroxide solution and extracted with 5 volumes of dichloromethane. The organic phase was dried over anhydrous sodium sulphate and the filtrate

was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 40 μl of ethyl acetate–HFBA (1:1, v/v) and derivatized at 70°C for 30 min.

To determine TIQ in wine (20 ml), beer (20 ml), whisky (20 ml) and milk (20 ml), the beverage was spiked with 50 ng of a mixture of [$^2H_6$]TIQ and [$^2H_7$]TIQ as an internal standard, and then 5 volumes of 0.4 *M* perchloric acid containing EDTA (0.1%, w/v) and ascorbic acid (0.1%, w/v) were added. The solution was worked up in a similar manner to the above.

Gas chromatography–mass spectrometry

A Shimadzu GC-9A gas chromatograph combined with a double-focusing mass spectrometer (Shimadzu 9020-DF) was used. The gas 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 to 160°C at 3°C/min. Electron-impact ionization energy of 70 eV, ion source temperature of 250°C, trap current of 60 μA and accelerating voltage of 3 kV.

Determination of TIQ by GC–MS

The TIQ levels in various foods were determined by selected ion monitoring (SIM). The mass numbers used for SIM were *m/z* 328 ([M−H]$^+$ ion of HFB-derivatized TIQ), *m/z* 329 (M^+ ion of HFB-derivatized TIQ), *m/z* 335 (M^+ ion of HFB-derivatized [$^2H_6$]TIQ) and *m/z* 336 (M^+ ion of HFB-derivatized [$^2H_7$]TIQ). A calibration line relating the concentration of TIQ to the peak-height ratio of TIQ at *m/z* 329 to the internal standard ([$^2H_6$]TIQ) at *m/z* 335 was obtained from the SIM chromatograms. The correlation coefficient of the calibration line for concentrations of TIQ ranging from 5 to 100 ng in 20 ml was 0.9984. The detection limit of TIQ using this method was 0.25 ng/ml.

RESULTS

Fig. 1 shows the EI mass spectra of (a) HFB-derivatized TIQ and (b) the HFB-derivatized mixture of [$^2H_6$]TIQ and [$^2H_7$]TIQ, which was used as an internal standard. Fig. 2 shows the mass chromatograms of (a) HFB-derivatized TIQ and (b) the HFB-derivatized extract from milk. Peak 1 in Fig. 2b was identified as TIQ, as it showed an identical retention time, the characteristic mass numbers (*m/z* 210 and 329) and an ion intensity ratio identical with those of authentic TIQ.

Fig. 3 shows the SIM chromatograms of (a) HFB-derivatized TIQ and HFB-derivatized extracts from (b) cheese, (c) milk and (d) the white of boiled egg. The mixture of [$^2H_6$]TIQ and [$^2H_7$]TIQ shows peaks at *m/z* 335 and 336 on the SIM chromatograms with a retention time of 7.6 min, and with an ion intensity ratio of *m/z* 335 to *m/z* 336 of 1:1. TIQ was detected in the foods as peaks at *m/z* 329 and 328 on the SIM chromatograms with retention times a

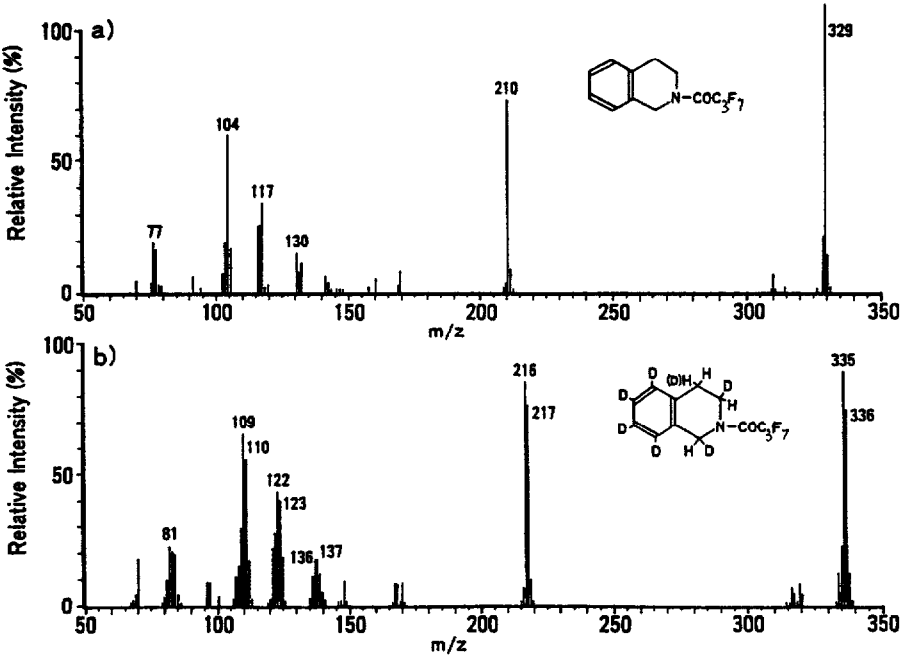

Fig. 1. EI mass spectra of (a) HFB-derivatized TIQ and (b) the HFB-derivatized mixture of $[^2H_6]$TIQ and $[^2H_7]$TIQ.

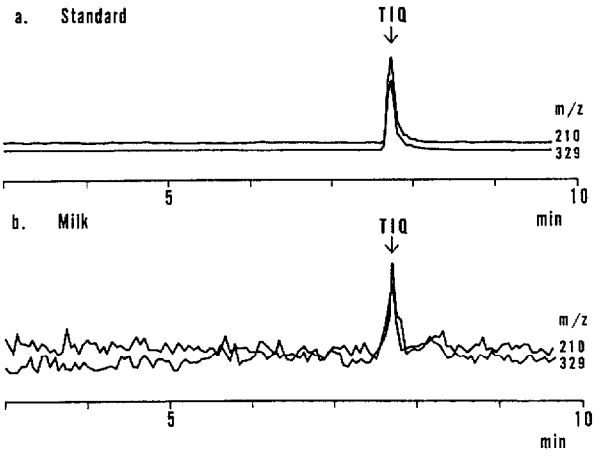

Fig. 2. Mass chromatograms of (a) HFB-derivatized TIQ and (b) the HFB-derivatized extract from milk.

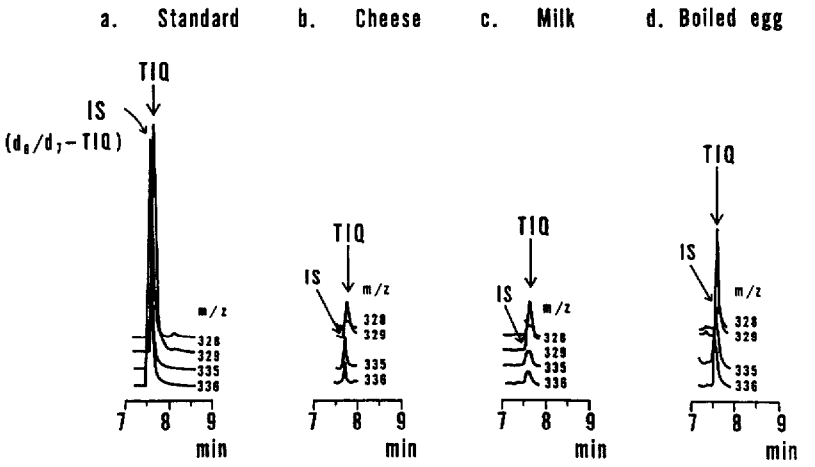

Fig. 3. SIM chromatograms of (a) HFB-derivatized TIQ and the HFB-derivatized extracts from (b) cheese, (c) milk and (d) the white of boiled egg.

TABLE I

CONCENTRATIONS OF TIQ IN FOODS

Sample	Concentration of TIQ (ng/g)	Sample	Concentration of TIQ (ng/g or ng/ml)
Cheese	5.2	Yolk of boiled egg	1.8
Banana	2.2	White of boiled egg	2.2
Broiled sardine	0.96	Wine	0.56
Broiled beef	1.3	Beer	0.36
Flour	0.52	Whisky	0.73
		Milk	3.3

few seconds later than deuterated TIQ, and with intensity ratio of *m/z* 329 to *m/z* 328 of 1:0.2.

The concentrations of TIQ in various foods are shown in Table I as the means of two or three samples. TIQ was present at high concentrations especially in cheese, milk, the white of boiled egg, banana and the yolk of boiled egg.

DISCUSSION

We detected TIQ in the various foods studied. Makino et al. [16] reported that TIQ was detected in cheese, wine and cocoa. We confirmed their findings, except for the presence of TIQ in cocoa, which could not be detected because of impurities in the extract.

TIQ has been detected in parkinsonian and normal human brains [12,13]. This TIQ could be derived from foods, as TIQ can easily pass through the blood–brain barrier [14] and may accumulate in the brains over a long period. The effects of its accumulation in the brain in relation to Parkinson's disease in humans remain for further study. It should be noted that the concentrations of TIQ in foods are very low in comparison with the very high concentration of TIQ (200 μg/g) in monkey brains needed to produce parkinsonian symptoms [14]. Therefore, TIQ in foods may not be related to the occurrence of Parkinson's disease.

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