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MIGRATION OF TETRAHYDROISOQUINOLINE, A POSSIBLE PARKINSONIAN NEUROTOXIN, INTO MONKEY BRAIN FROM BLOOD AS PROVED BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

1,2,3,4-Tetrahydroisoquinoline (TIQ) was quantitated by use of gas chromatography–mass spectrometry in brains and livers of marmosets which showed parkinsonism after daily subcutaneous injection of TIQ. TIQ showed greatly increased levels in the brains and livers of the TIQ-treated marmosets, with no detectable metabolites of TIQ. TIQ was present as an endogenous amine in the brains and livers of saline-treated marmosets at very low concentrations. It thus seems that TIQ can pass easily through the blood–brain barrier but cannot be metabolized in the brain or the liver. It is possible that TIQ accumulated in the brain may produce parkinsonism.

INTRODUCTION

Since a highly selective, irreversible neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that produces parkinsonism in humans, monkeys and mice[1–4] was discovered, an extensive search has been undertaken for endogenous or exogenous compounds that induce Parkinson's disease.

We screened of various compounds structurally related to MPTP for neurotoxicity by assaying the inhibition of the tyrosine hydroxylase system in tissue slices of the striatum[5–7]. After this screening, 1,2,3,4-tetrahydroisoquinoline (TIQ) and N-methyl-1,2,3,4-tetrahydroisoquinoline (N-Me-TIQ) were suggested as possible

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endogenous neurotoxins[8–10]. TIQ has been recently discovered in rat brain[11], and in the parkinsonian and normal human brains[12]. Repeated administration of TIQ to marmosets produced parkinsonian symptoms with reduction in tyrosine hydroxylase, dopamine and total biopterin concentrations in the substantia nigra[13]. To determine whether subcutaneously administered TIQ can pass through the blood–brain barrier, and whether TIQ can be metabolized in the brain and liver, we analysed the brains and livers of the TIQ-treated marmosets by use of gas chromatography–mass spectrometry (GC–MS).

EXPERIMENTAL

Chemicals

TIQ was purchased from Wako, and heptafluorobutyric anhydride (HFBA) from Gasukuro Kogyo.

A mixture of 1,3,4-trideutero-1,2,3,4-tetrahydroisoquinoline ([$^2H_3$]TIQ) and 1,3,4,4-tetradeutero-1,2,3,4-tetrahydroisoquinoline ([$^2H_4$]TIQ) was synthesized by a modified method of Wedekind and Oechslen[14]. Isoquinoline (1.29 g, 10 mmol) was dissolved in 37% deuterium chloride (15 g, deuterium content 99%) and stirred with tin powder at 60°C for 8 h, until the absorption at 325 nm had disappeared. The excess 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 choroform. The chloroform solution was then extracted with 2 *N* hydrochloric acid, and the extract was evaporated to dryness. The residue, after treatment with ethanol, gave the product as small prisms, 244 mg (18% yield), m.p. 174–177°C with decomposition. The synthesized compound was found to be a mixture of [$^2H_3$]TIQ (47.5%), [$^2H_4$]TIQ (47.5%), and TIQ (5%) by GC–MS. The structures of [$^2H_3$]- and [$^2H_4$]TIQ was assigned by ^1H NMR spectroscopy.

All the other chemicals used were of analytical grade.

Samples

Brains and livers were obtained from four marmosets[13]: A, a 2.1-year-old male weighing 435 g; B, a 2.8-year-old female weighing 470 g; C, a 2.5-year-old male weighing 370 g; and D, a 2.6-year-old female weighing 530 g. Saline alone was injected subcutaneously once a day for 16 days into A and B; TIQ was injected subcutaneously at a dose of 50 mg/kg once a day for 16 days into C and D. TIQ (1 g) was suspended in 20 ml of saline. Since TIQ does not dissolve in saline, the suspension was made by shaking the container just before injection. For GC–MS analysis, all four marmosets were sacrificed under deep ketamine-induced anesthesia on the 16th day of daily injection of either saline or TIQ. Brains and livers were immediately removed, and kept at −80°C until GC–MS analysis could be performed.

Sample preparation

To quantitate the TIQ levels in the brains and livers of TIQ-treated marmosets, tissue (0.5 g) was spiked with 50 μg of a mixture of [$^2H_3$]TIQ and [$^2H_4$]TIQ as an internal standard, and homogenized with 0.4 *N* perchloric acid (10 ml) containing EDTA (0.1% w/v) and ascorbic acid (0.1% w/v). To quantitate the TIQ levels in the brains and livers of saline-treated marmosets, tissue (0.5 g) was spiked with 50 ng of a

mixture of [$^2H_3$]TIQ and [$^2H_4$]TIQ as an internal standard and then homogenized likewise. To profile the amines in the brain and liver, the tissue sample was homogenized without addition of deuterated TIQ. The homogenate was centrifuged at 12 000 *g* for 15 min at 4°C. The supernatant was transfered to a glass test-tube and the pellet was vortexed with 0.4 *N* perchloric acid (10 ml) containing EDTA (0.1%) and ascorbic acid (0.1%) and centrifuged again. The combined supernatant was extracted with diethyl ether (10 ml). The aqueous phase was adjusted to pH 11 with 6 *N* sodium hydroxide and extracted twice 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 in ethyl acetate–HFBA (20 μl:20 μl), and derivatized at 70°C for 30 min.

Gas chromatography–mass spectrometry

A Shimadzu GC-9A gas chromatograph combined with a double-focusing mass spectrometer (Shimadzu 9020-DF) was used. 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 60μA, and an accelerating voltage of 3 kV.

Quantification of TIQ by GC–MS

To quantitate TIQ in the brains and livers of TIQ-treated marmosets, mass chromatography was carried out using 50 μg of a mixture of [$^2H_3$]TIQ and [$^2H_4$]TIQ as an internal standard, and the molecular ions of TIQ (*m/z* 329) and [$^2H_3$]TIQ (*m/z* 332) were monitored. A calibration line relating the concentration of TIQ to the peak-area ratio of TIQ at *m/z* 329 to the internal standard ([$^2H_3$]TIQ) at *m/z* 332 was obtained from the mass chromatograms. The correlation coefficient of the calibration line for concentrations of TIQ ranging from 10 μg to 500 μg per 0.5 g of tissue was 0.9997.

To quantitate TIQ in the brains and livers of saline-treated marmosets, selected-ion monitoring (SIM)was performed using 50 ng of a mixture of [$^2H_3$]TIQ and [$^2H_4$]TIQ as an internal standard. A calibration line relating the concentration of TIQ to peak area ratio of TIQ at *m/z* 329 to the internal standard ([$^2H_3$]TIQ) at *m/z* 332 was obtained from the SIM chromatograms. The correlation coefficient of the calibration line for concentrations of TIQ ranging from 5 ng to 300 ng per 0.5 g of tissue was 0.9987.

RESULTS

Fig. 1 shows the gas chromatograms of the HFB-derivatized extracts from the brains of TIQ-injected marmosets (a), and saline-injected marmosets (b). Deuterated TIQ was not added to the tissue homogenate. The EI mass spectrum of peak 1 in Fig. 1a is shown in Fig. 2a. The EI mass spectrum of the HFB-derivatized mixture of [$^2H_3$]TIQ and [$^2H_4$]TIQ used as an internal standard is shown in Fig. 2b. Peak 1 was identified as TIQ, since the peak showed a retention time and an EI mass spectrum

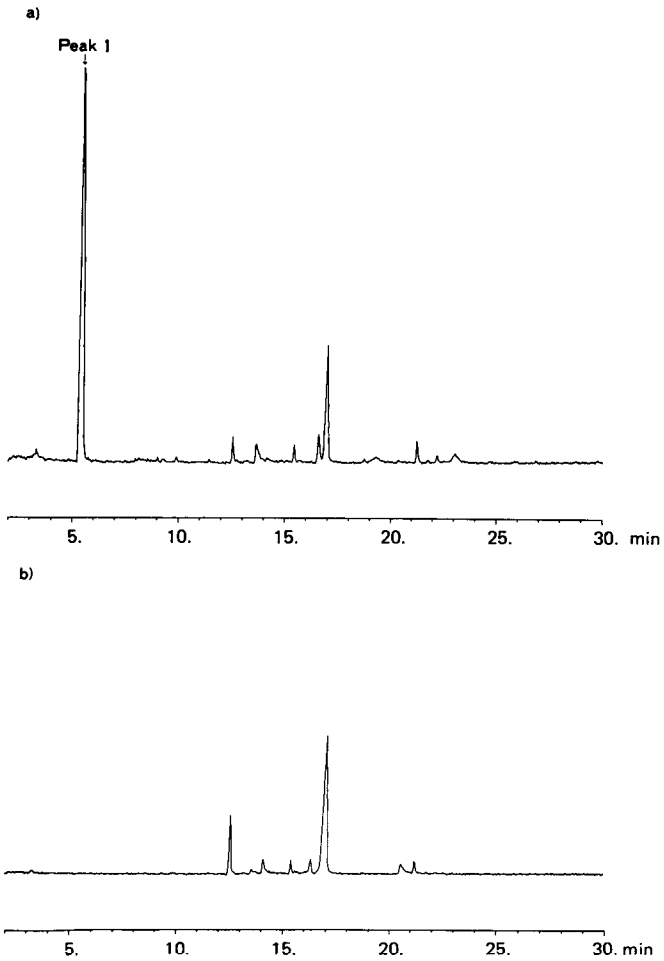

Fig.1. Gas chromatograms of the HFB-derivatized extacts from the brains of TIQ-treated marmosets (a), and saline-treated marmosets (control) (b). Deuterated TIQ was not added to the tissue homogenate. Peak 1 was identified as TIQ. The molecular ion of TIQ (*m/z* 329) was monitored by mass chromatography.

identical with those of HFB-derivatized TIQ. TIQ was detected in the brains of TIQ-treated marmosets at a high concentration, but no TIQ metabolites were detected.

Fig. 3 shows the gas chromatograms of the HFB-derivatized extracts from the livers of TIQ-treated marmosets (a) and saline-treated marmosets (b). TIQ was also detected in the livers of TIQ-treated marmosets at a high concentration, but no TIQ metabolites were detected.

Table I shows the concentrations of TIQ in the brains and livers of saline-injected and TIQ-injected marmosets. The concentrations of TIQ were greatly increased in the brains and livers of TIQ-treated marmosets. However, TIQ was detected in the brains and livers of saline-treated marmosets at low concentrations.

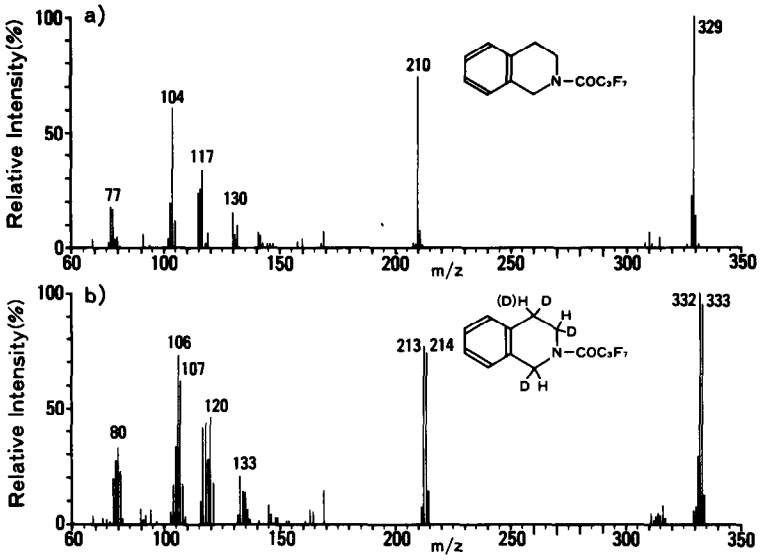

Fig. 2. EI mass spectra of peak 1 in Fig. 1a (a), and the HFB-derivatized mixture of $[^2H_3]$TIQ and $[^2H_4]$TIQ used as an internal standard (b).

DISCUSSION

We have reported the behavioural changes in the marmosets by the administration of TIQ[13]. The TIQ-injected marmosets showed features of parkinsonism, such as akinesia, tremor, and hypertonus. However, the saline-treated marmosets did not show any behavioural change at all. On the 8th day of TIQ injection, the legs of marmoset C had a slight tendency to drag. On the 11th day, a similar behavioural change was observed in marmoset D. The analysis of video recordings of the behavioural changes showed akinesia and hypertonus, which were more severe in marmoset C than in D. However, tremors in arms, legs, and trunk were more severe in D than in C. On the whole, the parkinsonian behavioural changes were more marked in marmoset C than in D. These results can be explained by our observation that the TIQ level in the brain of marmoset C was much higher than in marmoset D.

The concentration of TIQ in the brains of TIQ-injected marmosets was greatly increased, to the same degree as in the livers. This means that TIQ can pass easily through the blood–brain barrier. No metabolites of TIQ, such as 1,2-dihydroisoquinoline, could be detected in the brains or livers of TIQ-treated marmosets. This suggests that TIQ is not a substrate of monoamine oxidase. This result was also confirmed by our *in vitro* studies on TIQ metabolism in rat brains. The incubation of deuterated TIQ with mitochondria fraction or microsome fraction obtained from rat brain did not produce any metabolite of deuterated TIQ according to GC–MS analysis of the incubated medium using the same extraction method as from the marmosets' tissues.

TIQ was also detected as an endogenous amine in the brains and livers of control marmosets at low concentrations. However, there is an argument against the

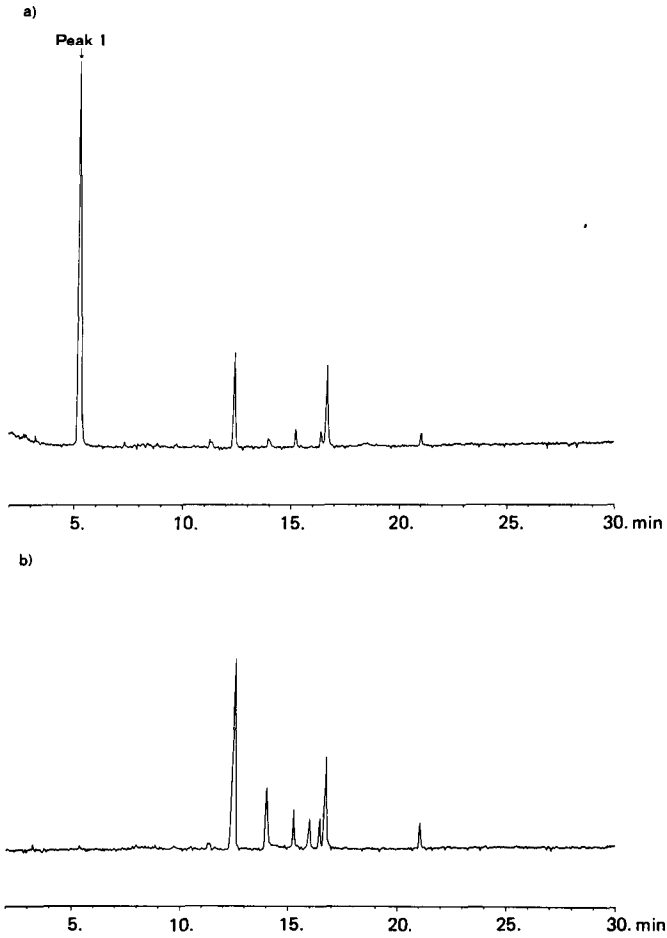

Fig. 3. Gas chromatograms of the HFB-derivatized extracts from the livers of TIQ-treated marmosets (a) and saline-treated marmosets (control) (b). Deuterated TIQ was not added to the tissue homogenate. Peak 1 was identified as TIQ. The molecular ion of TIQ (*m/z* 329) was monitored by mass chromatography.

TABLE I

CONCENTRATION OF TIQ IN BRAINS AND LIVERS OF MARMOSETS INJECTED WITH SALINE OR TIQ

Monkeys	*Concentration of TIQ (μg/g wet tissue)*	
	Brain	*Liver*
Saline (control)		
A	0.149	0.193
B	0.193	0.180
TIQ		
C	201	296
D	149	142

endogenous origin of TIQ, since TIQ could be artifactually formed from N-methylene-phenylethylamine by heptaflorobutyric acid during HFB derivatization (Dr. Boulton, personal communication). We are now investigating whether or not the endogenous TIQ is such an artifact, which may exist endogenously in tissues during the derivatization.

TIQ injected into marmosets could not be metabolized in the liver or brain, passed easily through the blood–brain barrier, then accumulated in the brain, and produced parkinsonian symptoms with reductions of dopamine and biopterin concentrations, and tyrosine hydroxylase activity[13]. Thus, TIQ may be an important endogenous amine that was demonstrated for the first time to produce Parkinson's disease in monkeys. Our results suggest that TIQ, an endogenous neurotoxin, is a candidate for causing Parkinson's disease in human beings.

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