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## Note

# Endogenous synthesis of N-methyl-1,2,3,4-tetrahydroisoquinoline, a precursor of N-methylisoquinolinium ion, in the brains of primates with parkinsonism after systemic administration of 1,2,3,4-tetrahydroisoquinoline

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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], structurally similar compounds that accumulate in the brain and induce parkinsonism have been extensively researched. 1,2,3,4-Tetrahydroisoquinoline (TIQ) was detected in the human brain [4], and subcutaneous injection of TIQ produced parkinsonism in primates with decreased amounts of dopamine and biopterin, and reduced activity of tyrosine hydroxylase [tyrosine,tetrahydropteridine:oxygen oxidoreductase (3-hydroxylating), EC 1.14.16.2] in the nigrostriatal regions [5].

Besides TIQ, N-methyl-1,2,3,4-tetrahydroisoquinoline (NMTIQ) was suggested to be a possible neurotoxin after screening of various compounds structurally related to MPTP for neurotoxicity [6]. NMTIQ is oxidized to the N-methyliso-

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quinolinium ion ($NMIQ^+$) by monoamine oxidase [monoamine:oxygen oxidoreductase (deaminating), EC 1.4.3.4] in human brain synaptosomal mitochondria [7]. $NMIQ^+$ inhibits tyrosine hydroxylase, the rate-limiting step of dopamine synthesis, in rat striatal slices [8], monoamine oxidase [9] and aromatic L-amino acid decarboxylase (aromatic L-amino acid carboxy-lyase, EC 4.1.1.18) [10]. These findings suggest that $NMIQ^+$ could be a potent endogenous neurotoxin similar to 1-methyl-4-phenylpyridinium ion ($MPP^+$), an oxidation product of MPTP formed by monoamine oxidase [11]. NMTIQ is proposed as an intermediate of the biosynthesis of $NMIQ^+$ from TIQ in the brain, and N-methylation of TIQ into NMTIQ has been reported to be produced *in vitro* by a N-methyltransferase in the human brain [12]. However, there has been no *in vitro* evidence that demonstrates the endogenous synthesis of NMTIQ from TIQ in the brain.

In this study we demonstrated, by detecting NMTIQ in monkey brain using gas chromatography–mass spectrometry (GC–MS), that the endogenous synthesis of NMTIQ from TIQ took place *in vivo* in the brain of the TIQ-injected monkey.

## EXPERIMENTAL

### *Materials*

Brains were obtained from four marmosets [5]: (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 sixteen days into A and B; TIQ (1 g suspended in 20 ml of saline) was injected subcutaneously at a dose of 50 mg/kg once a day for sixteen days into C and D. For GC–MS analysis, all four marmosets were killed under deep ketamine-induced anaesthesia on the sixteenth day of daily injection of either saline or TIQ. Brains were immediately removed and kept at −80°C until analysis.

TIQ was purchased from Aldrich. NMTIQ was synthesized by reduction in hydrogen chloride from N-methylisoquinolinium iodide. A mixture of 1,3,4-trideutero-N-methyl-1,2,3,4-tetrahydroisoquinoline ($d_3$-NMTIQ) and 1,3,4,4-tetradeutero-1,2,3,4-tetrahydroisoquinoline ($d_4$-NMTIQ) was synthesized by reduction in deuterium chloride from N-methylisoquinolinium iodide. N-Methylisoquinolinium iodide (1.075 g, 4 mmol) was dissolved in 37% deuterium chloride (10 g, 99% d) and stirred with 5 g of tin powder at 60°C. The reaction was monitored by absorbance at 330 nm. After 2 h the retention was complete. The excess tin was removed by filtration, and the filtrate was evaporated to dryness. The residue was taken up in water, made alkaline (pH 11) with sodium hydroxide and extracted with chloroform. The chloroform layer was washed with water and then extracted with 5 *M* hydrochloride acid. The extract was then evaporated to dryness, and the residue was crystallized from ethanol, to give the product (245 mg, 33.3% yield, m.p. 225–226°C). THe synthesized compound was

found to be a mixture of $d_3$-NMTIQ and $d_4$-NMTIQ by GC–MS and $^1H$ NMR spectroscopy. All other chemicals used were of analytical grade.

*Sample preparation*

To quantitate the NMTIQ levels in the brains of TIQ-treated marmosets, a brain sample (0.5 g) was spiked with 100 ng of a mixture of $d_3$-NMTIQ and $d_4$-NMTIQ as an internal standard and homogenized with 0.4 *M* perchloric acid (10 ml) containing EDTA (0.1%, w/v) and ascorbic acid (0.1%, w/v). To identify NMTIQ in the brain, the brain was homogenized without addition of $d_3$-NMTIQ and $d_4$-NMTIQ. 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 vortex-mixed with 0.4 *M* perchloric acid (10 ml) containing EDTA and ascorbic acid, and centrifuged again. The combined supernatant was extracted with diethyl ether (10 ml). The aqueous phase was adjusted to pH 11 with 6 *M* sodium hydroxide and extracted twice with dichloromethane (10 ml). The organic phase was extracted with 0.1 *M* hydrochloric acid (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* sodium hydroxide 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 in 20 μl of dichloromethane, and 6 μl of sample were analysed with GC–MS.

*Gas chromatography–mass spectrometry*

A Shimadzu GC-9A gas chromatograph connected to a double-focusing mass spectrometer (Shimadzu 9020-DF) was used. The chromatograph was equipped with a moving-needle type solventless injector, an OV-101 bonded fused-silica capillary column (25 m × 0.25 mm I.D.) or an NB-1 bonded fused-silica capillary column (50 m × 0.25 mm I.D.). The GC–MS conditions were: injection temperature, 330°C; column temperature programme, from 120 to 132°C at 1°C/min; separator temperature, 280 °C; ion source temperature, 250°C; the electron-impact (EI) ionization energy, 70 eV; trap current, 60 μA; accelerating voltage, 3.0 kV.

*Quantitation of NMTIQ by GC–MS*

To quantitate NMTIQ in the brains of TIQ-treated marmosets, selected-ion monitoring (SIM) was performed using 100 ng of a mixture of $d_3$-NMTIQ and $d_4$-NMTIQ as an internal standard. A calibration line relating the concentration of NMTIQ to the peak-height ratio of NMTIQ at *m/z* 146 to the internal standard ($d_3$-NMTIQ) at *m/z* 149 was obtained from the SIM chromatograms. The correlation coefficient of the calibration line for concentrations of NMTIQ ranging from 5 ng to 1000 ng per 0.5 g of tissue was 0.9975.

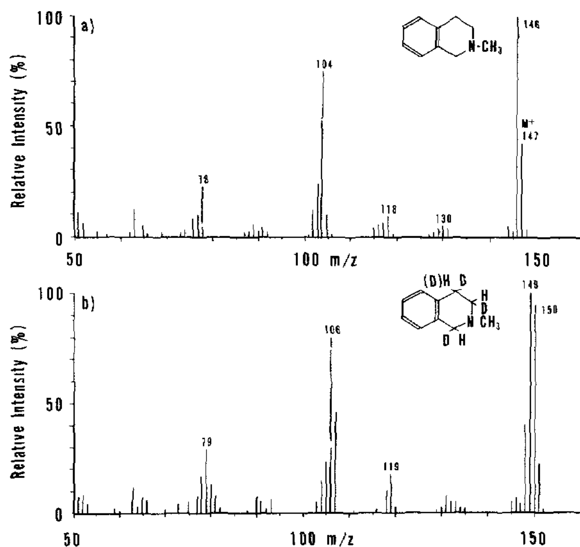


Fig. 1 EI mass spectra of (a) NMTIQ and (b) a mixture of $d_3$-NMTIQ and $d_4$-NMTIQ

RESULTS

Fig. 1 shows EI mass spectra of authentic NMTIQ (a) and a mixture of $d_3$-NMTIQ and $d_4$-NMTIQ (b). The EI mass spectrum of NMTIQ shows characteristic ions at *m/z* 147 ($M^+$) and 146. Using SIM with two different GC columns, NMTIQ was identified in the brains of TIQ-treated marmosets by the retention times and the characteristic selected mass numbers. Fig. 2 shows SIM chroma-

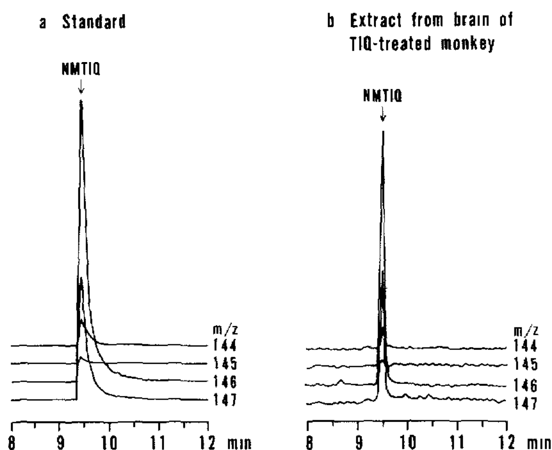


Fig. 2. SIM chromatograms of (a) authentic NMTIQ and (b) an extract from the brain of a TIQ-treated monkey, using an NB-1 capillary column.

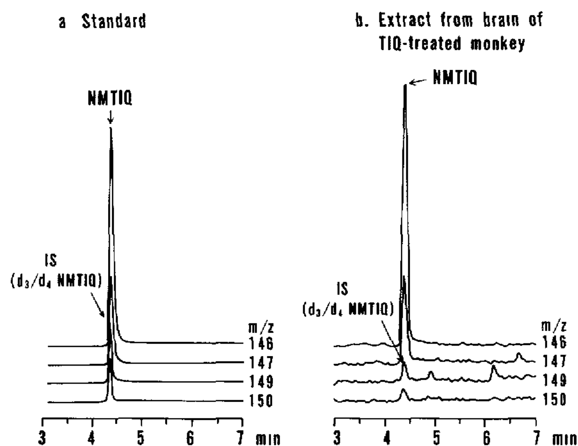


Fig. 3 SIM chromatograms of (a) authentic NMTIQ and a mixture of $d_3$-NMTIQ and $d_4$-NMTIQ and (b) an extract from the brain of a TIQ-treated monkey, using an OV-101 capillary column

tograms of authentic NMTIQ (a) and the extract from the brain of a TIQ-treated monkey (b), using an NB-1 capillary column. NMTIQ was detected only in the brain of the TIQ-treated monkey, since the peak showed identical retention time (9.5 min) and identical peak-height ratios (*m/z* 144/146, 145/146, 147/146) with those of authentic NMTIQ.

Fig. 3 shows SIM chromatograms of an authentic NMTIQ and a mixture of $d_3$-NMTIQ and $d_4$-NMTIQ (a), and the extract from the brain of a TIQ-treated monkey (b) using an OV-101 capillary column. The presence of NMTIQ was also confirmed in the extract from the brain of the TIQ-treated monkey, since the peak showed identical retention time (4.4 min) and identical peak-height ratio (*m/z*

TABLE I

CONCENTRATION OF NMTIQ IN THE BRAINS OF MARMOSETS INJECTED WITH SALINE OR TIQ

Marmosets A and B were controls injected with saline, and marmosets C and D were injected with TIQ for sixteen days The concentration of NMTIQ was measured as described in Experimental.

| Monkeys | Concentration of NMTIQ ($\mu$g/g wet weight) |
|---|---|
| *Injected with saline* | |
| A | Not detected |
| B | Not detected |
| *Injected with TIQ* | |
| C | 2 2 |
| D | 1 7 |

N-Methyltransferase → Monoamine oxidase

Tetrahydroisoquinoline (TIQ) → N-Methyltetrahydroisoquinoline (NMTIQ) → N-Methylisoquinolinium ion ($NMIQ^+$)

Fig 4 Metabolic pathway of TIQ in the brain.

147/146) with those of NMTIQ. A mixture of $d_3$-NMTIQ and $d_4$-NMTIQ was included as an internal standard in the extract of TIQ-treated monkey brain to quantitate the NMTIQ level.

The concentrations of NMTIQ in the brains of TIQ-treated marmosets and saline-treated marmosets are summarized in Table I.

## DISCUSSION

We detected NMTIQ in the brains of TIQ-injected monkeys, thus demonstrating the endogenous N-methylation of TIQ into NMTIQ, a precursor of the dopaminergic neurotoxin, $NMIQ^+$ (Fig. 4). Since the concentrations of TIQ in the brains of TIQ-injected monkeys were 201 $\mu g/g$ (C) and 149 $\mu g/g$ (D) [13], the concentrations of NMTIQ were *ca.* 1% of those of TIQ in the brains of TIQ-injected monkeys. The TIQ-injected monkeys showed parkinsonism symptoms, with decreased content of dopamine and biopterin and reduced activity of tyrosine hydroxylase in the substantia nigra [5]. NMTIQ detected in the brain could be further metabolized into $NMIQ^+$, which might have inhibited the catecholamine metabolism in the brain. $NMIQ^+$ is non-volatile, so that it is not detectable by GC–MS. Therefore, the use of liquid chromatography–mass spectrometry might be necessary to detect $NMIQ^+$ in the brain.

TIQ in parkinsonian and normal human brains has been demonstrated not to be an artifact formed during extraction or derivatization [14]. At present, TIQ detected in the human brain is considered to be derived from foods, since TIQ is present in various foods, such as cheese, milk, banana, boiled egg, boiled beef and sardine [15], and since TIQ can easily pass through the blood–brain barrier [13]. However, *in situ* biosynthesis of TIQ from formaldehyde and $\beta$-phenylethylamine cannot be excluded. NMTIQ may be present in the human brain at trace concentrations, since NMTIQ is an endogenous metabolite of TIQ as demonstrated in this study. We are now trying to detect NMTIQ in parkinsonian human brain.

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