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Determination of 1-methyl-1,2,3,4-tetrahydroisoquinoline in mouse brain after treatment with haloperidol by gas chromatography–selected ion monitoring

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Abstract

The content of the endogenous amine, 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ), in mouse brain, treated with the antipsychotic agent haloperidol (HP) was determined by GC–SIM (selected ion monitoring) system. 1-MeTIQ in brain was extracted with chloroform at pH 11–12 and was detected as PFP derivative by GC–SIM. The 1-MeTIQ contents in mouse brains following intraperitoneal administration of HP or its dehydrated product, HPTP (1 and 4 mg/kg per day, for four days), were markedly reduced compared with control groups. This result agrees well with the findings in human idiopathic parkinsonianism and in MPTP-treated mouse brain. In addition, this finding suggests that the change of the endogenous amine 1-MeTIQ content in the brain plays an important role in the pathogenesis of toxin-induced parkinsonism.

Keywords: Haloperidol; 1-Methyl-1,2,3,4-tetrahydroisoquinoline

1. Introduction

The endogenous amines 1,2,3,4-tetrahydroisoquinoline (TIQ, **1**) and 1-methyl-1,2,3,4-tetrahydroisoquinoline (1-MeTIQ, **2**) (Fig. 1) are structurally related compounds to the parkinsonian-inducing agent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **3**) and have been found in the brains of rodents and humans [1–4]. TIQ is well known to have neurotoxicity and to induce parkinsonism [5–11], however, the TIQ content in the parkinsonian brains was not significantly less than in the control brains. On the other hand, the 1-MeTIQ content was significantly less in the parkinsonian brain than in the control and tended to decrease with aging [4]. This result has been also confirmed in the rat brain [12]. Moreover, pretreatment with 1-MeTIQ in mice completely prevented MPTP- or TIQ-induced bradykinesia, one of the symptoms of parkinsonism [3].

Haloperidol, 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-4-piperidinol, (HP, **5**) (Fig. 1) is a widely used antipsychotic agent that causes often extrapyramidal side effects including acute dystonic reactions, akathisa, drug-induced parkinsonism, and following chronic treatment, tardive dyskinesia [13]. HP may undergo acid-catalyzed [14] and/or enzyme mediated [15] dehydration to produce 4-(4-

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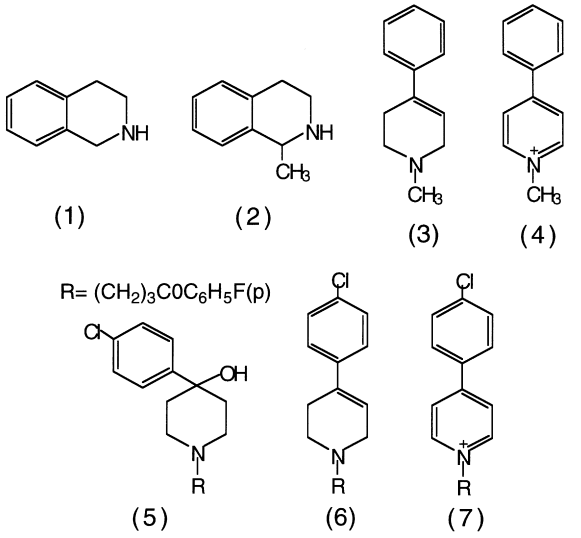

Fig. 1. Chemical structures discussed in this report.

chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]-1,2,3,6-tetrahydropyridine (HPTP, **6**), a compound that has structural features similar to those of MPTP. Moreover, the results from earlier studies have documented the oxidative biotransformation of HP and its dehydration product, HPTP, to the corresponding pyridinium metabolite, the 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]pyridinium ion (HPP^+, **7**), in rodents [16–18] and HP to HPP^+ in humans [19–21]. Intracerebral microdialysis [22,23] and neuronal cell culture [24,25] studies have shown that HPP^+ has neurotoxic properties resembling those of the 1-methyl-4-phenylpyridinium ion (MPP^+, **4**), derived from MPTP via monoamine oxidase B (MAO-B) [26] and cytochrome P450 [27]. These findings support the theory that the biotransformation of HP to HPP+ could be important in the extrapyramidal side effects derived from HP.

We have been interested in the possible role of the endogenous amine 1-MeTIQ in preventing the pathogenesis of parkinsonism derived from exogenous compounds. In the present study, to confirm that the 1-MeTIQ content in the brain decreases in the drug-induced parkinsonism model animals, we determined the endogenous 1-MeTIQ content in mouse brain treated with the antipsychotic agent HP using a more sensitive and selective method of GC–MS system. Analytical method has been reported only in the literature for the detection of 1-MeTIQ in rat brain using GC–MS [2]. This method requires a complex procedure for liquid–liquid extraction and is not suitable for routine analysis. So, we modified it in part and determined the 1-MeTIQ content in brain using this GC-MS method after administration of the drug.

2. Experimental

2.1. Chemicals and reagents

Haloperidol was purchased from Sigma Chemical (St. Louis, MO, USA), the hydrochloride of HPTP was synthesized in our laboratory [16], 1-MeTIQ was synthesized according to the procedure described previously [3], and pentafluoropropionic anhydride (PFPA), dibenzylamine and Tween 20 were obtained from Nakalai Tesque (Kyoto, Japan). All other chemicals and reagents were of analytical grade from commercial sources.

2.2. Sample preparation for measurement of 1-MeTIQ content in brain

Male ddY mice (25–28 g, SLC, Shizuoka, Japan) were injected with 1 or 4 mg/kg of HP or HPTP (20% Tween 20 in saline solution) intraperitoneally for four days. The mice were killed by decapitation at 24 h after the last injection and the brain was quickly removed. Each brain was weighed, placed in an ice-cooled Potter-Elvehjem type homogenizer, and homogenized in two volumes of 0.4 *M* perchloric acid, containing 0.05% (wt/vol) ascorbic acid, EDTA, and 0.02% (wt/vol) semicarbazide hydrochloride. The homogenate was centrifuged (12 000 *g* for 20 min at 4°C) and the supernatant was separated. Dibenzylamine (2 ng/ml in 0.1 *M* HCl) was added as an internal standard to the supernatant for quantitation. The mixture was adjusted to pH 11–12 with 1 *M* NaOH and then extracted with five volumes of chloroform. The organic phase was dried over anhydrous sodium sulfate and the filtrate evaporated to dryness. The residue was dissolved in ethyl

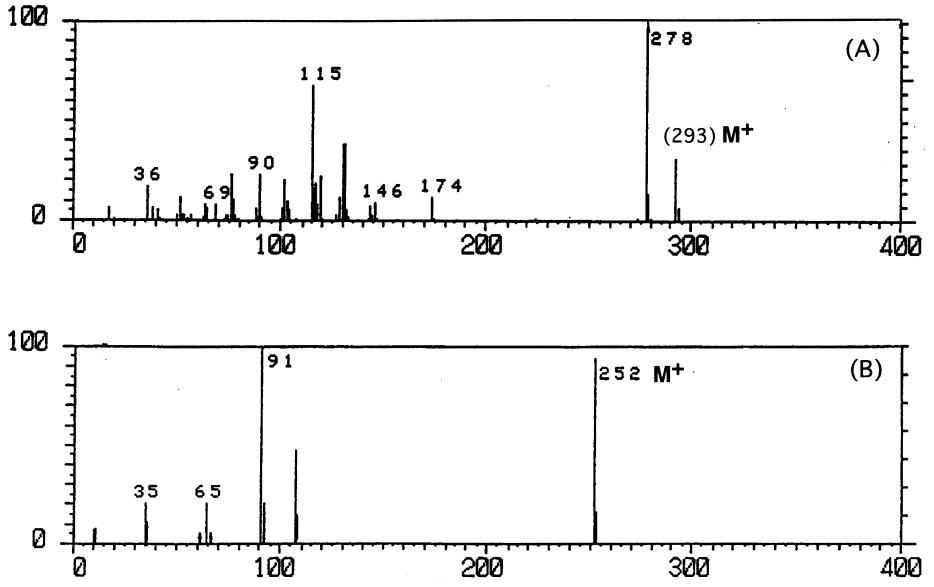

Fig. 2. Mass spectra of 1-MeTIQ (A) and dibenzylamine (I.S.) (B) as PFP derivatives.

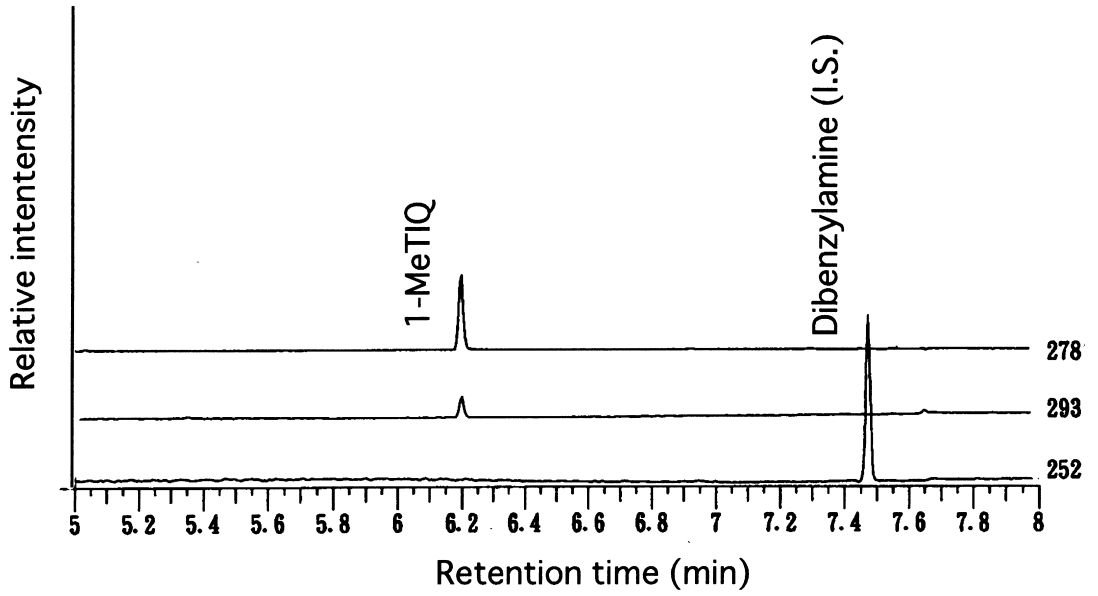

Fig. 3. SIM chromatogram of an extract from the mixture of 1-MeTIQ standard solution (2 ng/ml) and dibenzylamine (I.S.) as PFP derivatives. Chromatographic conditions: DB-1 capillary column, 30 m×0.25 mm I.D.; film thickness, 1.0 μm; column temperature, 70 (1 min) to 300°C (20°C/min); injection port, 250°C; interface, 280°C, He at 50 kPa; total flow-rate, 50 ml/min; electron impact mode at an ionization energy of 70 eV. A 1 μl aliquot of the samples was injected in the spiltless mode at a column temperature of 70°C, and the spilter was opened after 1 min.

acetate–PFPA (60:30 μl) and left in a water bath for 30 min at 70°C.

2.3. Gas chromatography–mass spectrometry analysis

GC–MS was carried out using a gas chromatograph (Hitachi G-3000, Tokyo, Japan) and a double-focusing mass spectrometer (Hitachi M-4000, Tokyo, Japan). A DB-1 capillary column (30 m× 0.25 mm I.D., film thickness 1.0 μm, J & W Scientific, Folsom, CA, USA) was inserted directly into the ion source, and each analysis was operated in the splitless mode with helium as the carrier gas. The oven temperature was held at 70°C for 1 min following injection and programmed to 300°C at a rate of 20°C/min. The injection port and interface temperatures were 250 and 280°C, respectively. The

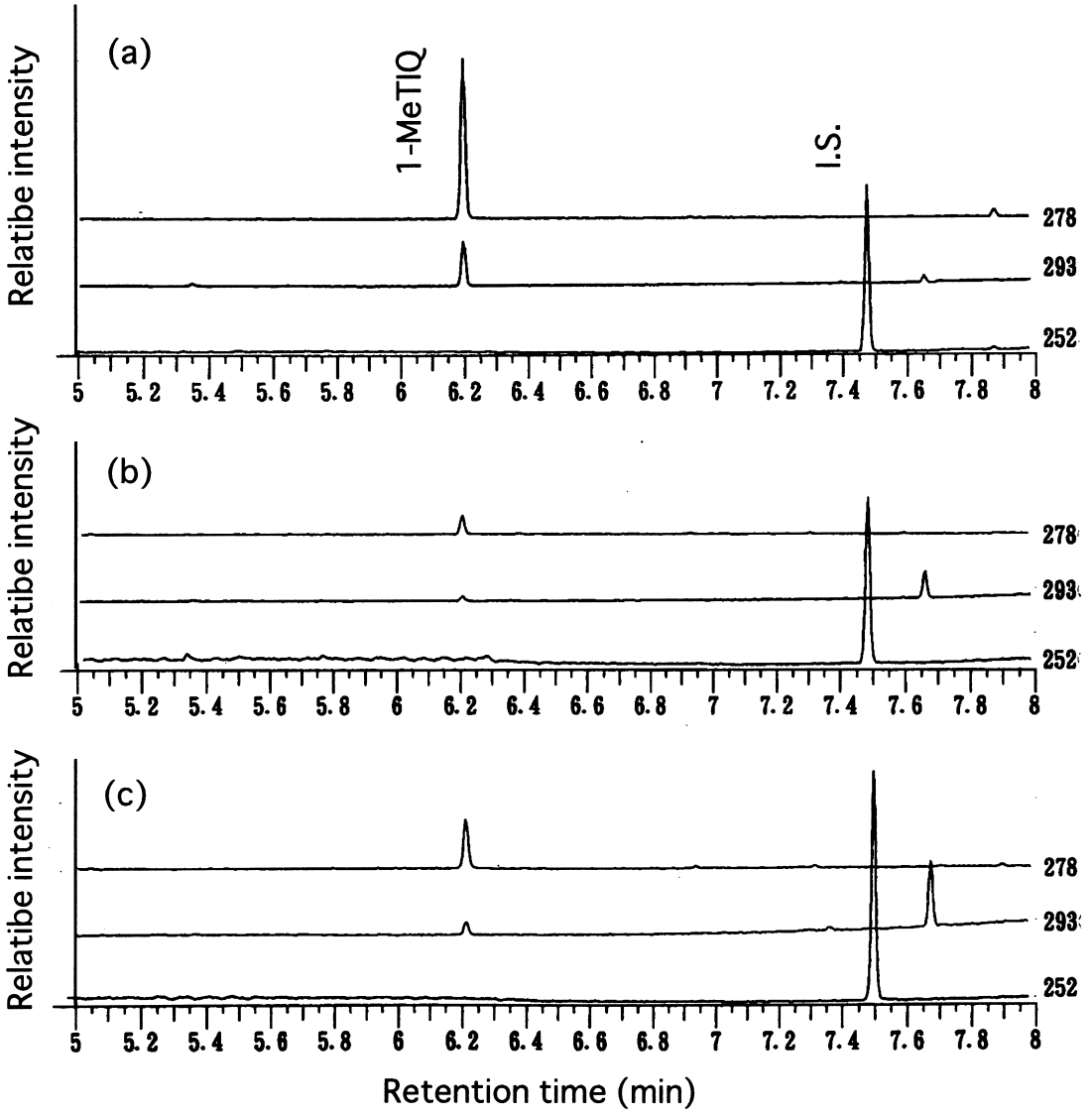

Fig. 4. SIM chromatogram of an extract obtained from (a) the control, (b) HP- and (c) HPTP-treated mouse brains as PFP derivatives. Both HP and HPTP were dosed intraperitoneally at 4 mg/kg for four days. Chromatographic conditions are as Fig. 3.

mass spectrometer was operated under electron impact mode at an ionization energy of 70 eV. The chosen diagnostic mass fragments (m/z) were monitored for the compound in selected ion monitoring (SIM) mode. The mass numbers used for characterization and quantification were m/z 278, 293 (PFP-derivatized 1-MeTIQ) and m/z 252 (PFP-derivatized dibenzylamine).

3. Results and discussion

The electron-impact mass spectra and the representative SIM chromatograms of PFP derivatives of authentic 1-MeTIQ and dibenzylamine (I.S.) are shown in Figs. 2 and 3. The mass spectra of authentic 1-MeTIQ and dibenzylamine gave characteristic fragment ions at m/z 293 (M^+), 278 (M^+-15), and at m/z 252 (M^+), respectively. The peaks of authentic 1-MeTIQ and I.S. were well separated and appeared in good shape in the SIM chromatograms using these characteristic fragment ions.

The calibration curve for 1-MeTIQ was obtained by plotting the peak area ratio of 1-MeTIQ (m/z 278) to I.S. (m/z 252) versus the amount of 1-MeTIQ. As a result, excellent linearity was observed over the concentration range examined (0.5–50 ng/ml for standard solution, $y=0.119x-0.018$, $r^2=0.999$). The calibration curve showed little day-to-day variability in slopes and intercepts [coefficient of variation (C.V.), <6%]. The lower limit of detection was approximately 0.1 ng/ml for standard solution. Experiments with spiked samples resulted in a recovery of 92.9±3.1% at a concentration of 10 ng/g of tissue (data not shown).

The endogenous 1-MeTIQ content in mouse brain was determined by SIM. Fig. 4a shows the SIM chromatograms of an extract from the control mouse brain. The peak of 1-MeTIQ was detected at a retention time of 6.2 min. The chromatogram was free of interference at the retention times of interest. The SIM chromatograms of extracts from HP (4 mg/kg)- and HPTP (4 mg/kg)-treated mouse brains were shown in Figs. 4b and c. It is apparent that the peak height of 1-MeTIQ in both extracts was decreased markedly compared that in control. The endogenous 1-MeTIQ contents in control, HP- and H PTP-treated mouse brains were shown in Fig. 5.

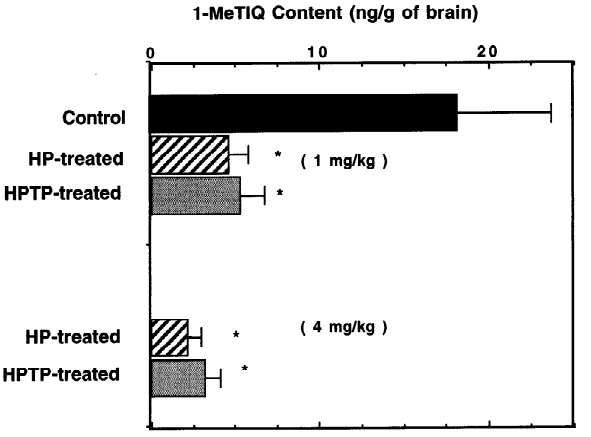

Fig. 5. 1-MeTIQ contents in control, HP- and HPTP-treated mouse brain. Both HP and HPTP were dosed intraperitoneally at 1 and 4 mg/kg for 4 days. *Significantly different from the corresponding controls ($P<0.01$, Student's t-test).

Whereas the 1-MeTIQ content in brain tissue was 18.3±5.1 ng/g of tissue in control mice, those in HP- and HPTP-treated mice were 4.6±1.2 at 1 mg/kg and 2.2±0.8 at 4 mg/kg, and 5.3±1.4 at 1 mg/kg and 3.2±0.9 ng/g of brain tissue, respectively.

The significant decrease of the endogenous amine, 1-MeTIQ, contents in the HP- and HPTP-treated mouse brains in comparison with that in control brain agrees well with the previous finding of MPTP-treated mice [3]. We have been very interested in these results because 1-MeTIQ content was reduced in parkinsonian brains [4], and pretreatment with 1-MeTIQ perfectly prevented mice treated with MPTP from bradykinesia, one of the characteristic symptoms in Parkinson's disease [3]. It is unclear why the 1-MeTIQ content in brain is reduced following treatment with MPTP-like compounds. It is possible that HP, HPTP, and MPTP may inhibit the enzymatic formation of 1-MeTIQ in brain from 2-phenylethylamine and acetaldehyde. If the use of the endogenous amine 1-MeTIQ plays an important role in the pathogenesis of parkinsonism derived from exogenous compounds, 1-MeTIQ may become a possible leading compound of anti-parkinsonism agents.

In conclusion, the endogenous amine 1-MeTIQ in brain was determined by the combination of simple extraction, PFP-derivatization and the use of GC–

SIM system. The 1-MeTIQ contents in HP- and HPTP-treated mouse brains were decreased markedly in comparison with that in the control.

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