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TETRAHYDROISOQUINOLINE AND 1-METHYL-TETRAHYDROISOQUINOLINE AS NOVEL ENDOGENOUS AMINES IN RAT BRAIN

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SUMMARY: This is the first report to identify 1,2,3,4-tetrahydroisoquinoline and 1-methyl-1,2,3,4-tetrahydroisoquinoline as endogenous amines from non-treated rat brain. The detection was performed by coupled gas chromatography - multiple ion detection, using heptafluorobutyric anhydride and pentafluoropropionic anhydride as derivatizing reagents. These amines might be endogenous substances inducing parkinsonism.

Some tetrahydroisoquinoline alkaloids have been detected as endogenous compounds in plants (1) and in animals. For example, salsolinol(6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline) has been reported to exist in rat brain (2) and in the urine of parkinsonian patients on L-dopa treatment (3). It is suggested that these alkaloids play an important role as neuromodulators in the sense of fine tuning of the action of neurotransmitters (4). Furthermore, these alkaloids may cotribute to pathological conditions such as alcoholism and phenylketonuria (5). Especially, the relation of salsolinol to alcoholism has been discussed frequently (6-8).

On the other hand, 2-phenylethylamine (PEA) is one of the biogenic amines. It has been reported that its apparent turnover

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Abbreviations: TIQ, 1,2,3,4-tetrahydroisoquinoline; 1MeTIQ, 1-methyl-1,2,3,4-tetrahydroisoquinoline; 2MeTQ, 2-methyl-tetrahydroquinoline; PEA, 2-phenylethylamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; HFB, heptafluorobutyric; HFBA, HFB anhydride; PFP, pentafluoropropionic; PFPA, PFP anhydride; GC-MID, coupled gas chromatography - multiple ion detection.

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is very fast (9), so only a small amount of PEA can be detected in rat brain (10). Because of inactivation by monoamineoxidase (MAO), PEA has not yet been studied extensively in vivo. Chemically, PEA, which has no electron-donating substituents on the phenyl ring, can condense with formaldehyde by a Pictet-Spengler reaction to form 1,2,3,4-tetrahydroisoquinoline (TIQ) (11). It has been reported however, that the condensation of PEA and formaldehyde does not occur under physiological conditions (12).

Recently, it has been found that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is very remarkable as a substance inducing parkinsonism (13-14). Nagatsu's group has assumed that TIQ might be one of the endogenous substances inducing parkinsonism because of its structural similarity to MPTP and its possibility of existence in vivo (15).

If the existence of endogenous TIQ can be confirmed, TIQ becomes important as a pharmacologically active compound and an endogenous substance possibly inducing parkinsonism. In this paper, we report the detection of TIQ and 1-methyl-TIQ (1MeTIQ) in the brain of untreated rats. The two endogenous amines, which may be formed by the condensation of PEA with formaldehyde and acetaldehyde, respectively, were identified and their levels in the rat brain determined by coupled gas chromatography - multiple ion detection (GC-MID).

MATERIALS AND METHODS

Materials: TIQ, heptafluorobutyric anhydride (HFBA) and pentafluoropropionic anhydride (PFPA) were purchased from Wako Pure Chemical Industries, Ltd., 2-phenylethylamine (PEA) and 2-methyl-1,2,3,4-tetrahydroquinoline (2MeTQ) from Tokyo Chemical Industry Co., Ltd. 1MeTIQ was synthesized according to the literature (16). All other chemicals were of analytical purity.

Sample preparation: Male Wistar rats weighing 130-150g were anesthetized with ether. The brain was removed rapidly under ice-cooling, and homogenized in 0.4N perchloric acid (10ml) containing EDTA (0.1%w/v) and ascorbic acid (0.1%w/v). The homo-

genate was centrifuged (12,000g;15min;4°C) and the supernatant was separated. The pellet was treated again as mentioned above. The combined supernatants were washed with ether. The aqueous phase was adjusted to pH11.0 and extracted with CH_2Cl_2 (10mlx2). The organic phase was then extracted with 0.1N HCl solution (10ml) containing EDTA (0.1%w/v) and ascorbic acid (0.1%w/v). The acidic phase was adjusted to pH11.0 and extracted with CH_2Cl_2 (10mlx2). The organic phase was dried over anhydrous Na_2CO_3 and the filtrate evaporated to dryness. The residue was dissolved in ethyl acetate:HFBA (30µl:20µl) and left in a water bath for 30min at 70°C. Before the semiquantitation, a constant volume of the HFB derivatized 2MeTQ solution was added to every sample as an internal standard. The used mass numbers for semiquantitation were m/z329 (TIQ), m/z328 (1MeTIQ) and m/z343 (2MeTQ).

Gas chromatography - mass spectrometry: The GC-MS was carried out on a Jeol MS-GCG-05 Gas Chromatograph / JMS-DX300 Mass Spectrometer / JMA-200 Mass Data Analysis System. The column (2.5mmx2m) was packed with Shimalite (80-100mesh) coated with Silicone OV-17 (3%). The flow rate of the carrier gas (He) was 28-30ml/min. The column temperature was 175°C, injection temperature 190°C, electron energy 70eV and ionization current 100µA.

RESULTS AND DISCUSSION

The electron impact mass spectra of the HFB derivatives of authentic TIQ and 1MeTIQ show characteristic fragment peaks at m/z 329 (M^+), m/z 210, and at m/z 343 (M^+), m/z 328, respectively (Fig. 1).

Using Multiple ion detection (MID), TIQ and 1MeTIQ were identified by their retention times and their characteristic selected mass numbers (Fig. 2). In oder to prevent interference for biological impurities, the ion intensity ratio (m/z 329/210) for TIQ was measured, and found to be idetical to the ratio of the authentic TIQ (Fig. 3). Moreover, the detection of TIQ and 1MeTIQ were confirmed by using a different derivatizing reagent, PFPA instead of HFBA. This experiment showed that the observed peaks do not include impurities (data not shown). Semiquantitation of TIQ and 1MeTIQ from non-treated rat brain extracts show levels of 5-7 ng/g brain and 1-3 ng/g brain, respectively (Table 1).

The possible formation of TIQ and 1MeTIQ during the sample work-up procedure was checked by the addition of PEA-HCl to the

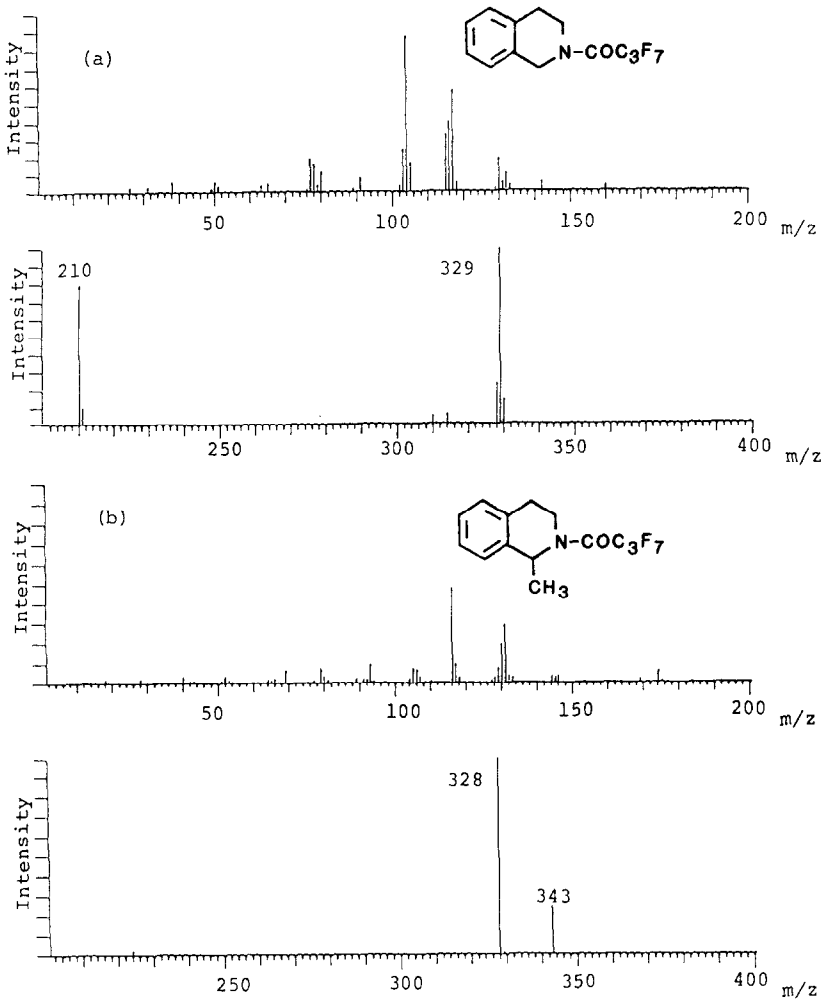


Figure 1. Mass spectra of the authentic samples of HFB derivatives; (a) TIQ, (b) 1MeTIQ.

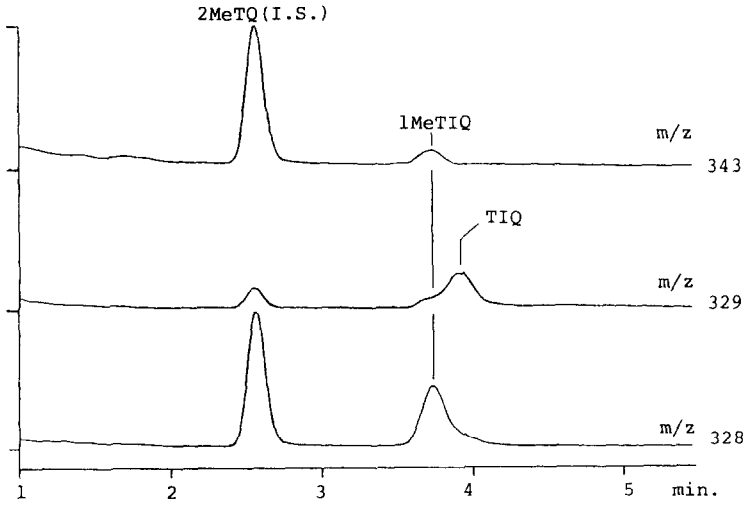


Figure 2. GC-MID chromatogram of a rat brain sample.

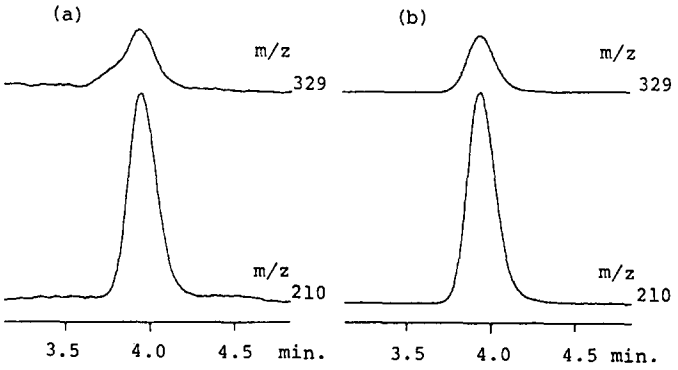


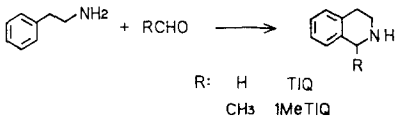
Figure 3. GC-MID chromatogram of TIQ- (a) rat brain and (b) satndard sample observed at m/z 329 and 210.

brain homogenate. It was reported that the amount of the endogenous PEA was 1.4 ng/g brain in male Sprague-Dawley rat (10). Hence, the amounts of PEA-HCl added were 10 ng and 100 ng. However, there was no intrinsic change of the TIQ and 1MeTIQ values upon the addition of PEA (Table 1).

The above results demonstrate that both TIQ and 1MeTIQ are normal constituents of rat brain. The proposed mechanism of TIQ and 1MeTIQ formation is shown in scheme 1. A similar mechanism has been suggested for the formation of different tetrahydroisoquinoline alkaloids (17). This condensation is facilitated by elctron-donating substituents (e.g. alkoxy or hydroxy groups) in appropriate positions of the aromatic ring (4). Dopamine, which has an effective substituent (meta-OH group) on the phenyl ring, can easily cyclize with aldehyde under physiological conditions.

Table 1. TIQ and 1MeTIQ levels in rat brain samples (run 1,2,3) and in samples, where PEA had been added

	TIQ (ng/g brain)	1MeTIQ (ng/g brain)
run 1	5.6	1.6
run 2	4.7	1.7
run 3	5.8	2.6
PEA added		
0 ng	6.7	0.7
10 ng	4.9	0.8
100 ng	5.3	0.7



Scheme 1. Proposed mechanism of TIQ and 1MeTIQ formation.

But, PEA, which has no effective substituents, does not cyclize under these conditions (12). Therefore if TIQ and 1MeTIQ are formed from PEA, it can be assumed that this reaction proceeds enzymatically.

TIQ is assumed to be one of the endogenous substances inducing parkinsonism. Like MPTP, the injection of TIQ to rat decreased the level of 3,4-dihydroxyphenylacetic acid, a dopamine metabolite, and the tyrosine hydroxylase activity (15). In this paper, we identified TIQ and 1MeTIQ as endogenous amines in non-treated rat brain. From this discovery, more information about their function under physiological as well as pathological conditions seems desirable. Their pharmacological effects are now under investigation.

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