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GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC EVIDENCE FOR THE IDENTIFICATION OF 6,7-DIHYDROXY-1,2,3,4-TETRAHYDROISOQUINOLINE AS A NORMAL CONSTITUENT OF RAT BRAIN

ITS QUANTIFICATION AND COMPARISON TO RAT WHOLE BRAIN LEVELS OF DOPAMINE*

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Abstract—Gas chromatographic/mass spectrometric data are presented which demonstrate the presence of 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (DHTIQ) as a normal constituent of rat brain. The level of DHTIQ was calculated to be 10.0 ± 3.0 ng/g wet weight (±S.D., N = 9) of brain tissue while the level of dopamine (DA) was measured as 1.22 ± 0.22 μg/g (N = 14). The ratio of DHTIQ:DA was thus observed to be approximately 1:100. The possible formation of DHTIQ in alcoholism and schizophrenia is discussed.

The formation of 1,2,3,4-tetrahydro-β-carbolines (THBCs) and 1,2,3,4-tetrahydroisoquinolines (TIQs) via the Pictet–Spengler condensation of biogenic amines with aldehydes has been demonstrated repeatedly in *in vitro* tissue preparations and biomimetic systems (for a review see Refs. 1–3). The formation of these compounds *in vivo* without concomitant pharmacological intervention has remained a matter for conjecture. However, several β-carbolines derived from the trace indoleamines and formaldehyde have been identified recently as naturally occurring products of tryptophan metabolism in man and other mammals [4–10]. The formation of 6,7-dihydroxy-TIQ (DHTIQ) from dopamine and formaldehyde has been observed to occur at a rate approximately 100 times greater than that for THBC formation from tryptamine and formaldehyde when reacted under biomimetic conditions [11]. Thus, one could speculate that the non-enzymatic formation of DHTIQ from dopamine (DA) and formaldehyde (HCHO), the most reactive of all aldehydes in the Pictet–Spengler reaction, should also occur in brain tissues and be present in greater amounts than the non-enzymatically formed β-carbolines derived from the trace indoleamines tryptamine (TA) and 5-methoxy-TA (5-MeOTA).

To examine this possibility we have developed a gas chromatographic/mass spectrometric (GC/MS) method for the simultaneous analysis of DA and DHTIQ in rat whole brain. In this report we present evidence for the identification of DHTIQ as a normal constituent of rat brain. A comparison of the whole brain levels of DHTIQ and DA is also presented.

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MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (200–300 g) were obtained from Southern Animal Farms, Prattville, AL, U.S.A. The rats were housed in groups of three and given food and water *ad lib*.

Materials

Paraformaldehyde-d_2 (99.8 per cent isotopic purity, Lot No. D-900) and α,α,β,β-tetradeuterodopamine·HCl (DDA) were obtained from Merck Sharpe & Dohme Isotopes, Montreal, Canada. Dopamine·HCl and Amberlite CG-50 were obtained from the Sigma Chemical Co., St. Louis, MO, U.S.A. 3,4-Dimethoxyphenethylamine·HCl (DMPEA) was provided by Professor Fred Benington and Dr. Richard Morin of this laboratory. Solvents were of spectro-grade and were obtained from Burdick & Jackson Laboratories, Inc., Muskegon, MI, U.S.A. All other chemicals were obtained from commercial sources and were of the highest available purity.

Preparation of DHTIQs

1,1-Dideutero-DHTIQ (D_2DHTIQ). The DMPEA·HCl (1.0 g, 4.6×10^{-3} moles) was dissolved in 5.0 ml of water containing 0.2 g of paraformaldehyde-d_2 (6.7×10^{-3} moles). Concentrated HCl (0.5 ml) was then added and the reaction mixture was refluxed for 3 hr. The mixture was cooled to room temperature and evaporated to give

a white crystalline solid. The solid was collected by suction filtration and washed with 50.0 ml of acetone to give 0.75 g of 1,1-dideutero-6,7-dimethoxy-TIQ·HCl. Yield = 71 per cent; m.p. = 240–242°; Lit. value = 241° [12].

The 1,1-dideutero-6,7-dimethoxy-TIQ · HCl (0.75 g, 3.27×10^{-3} moles) was dissolved in 20.0 ml of 48% HBr and refluxed for 2 hr. The solution was cooled to room temperature and evaporated to a volume of 10.0 ml. This was placed at 5° overnight to give white needles of D_2DHTIQ · HBr which were collected by suction filtration and washed with ice-cold acetone (20 ml). Yield = 0.72 g, 89.5 per cent; m.p. = 260–262°; Lit. value = 260–262° [13].

DHTIQ. The DHTIQ was prepared as described above using paraformaldehyde.

Preparation of samples for GC/MS analyses

Rats were decapitated and the brains were rapidly excised. Each brain was immediately placed in a tared homogenizing flask containing 14% $HClO_4$ and various internal standards, as listed below, for the different experiments.

Linearity of recovery and limits of detection of DA and DHTIQ. Three weighed rat brains were homogenized in 12.5 ml of 14% $HClO_4$ and brought to a volume of 25.0 ml with water. This was divided into five equal fractions, to which were added 2000 ng of DDA · HCl and 2000 ng of D_2DHTIQ · HBr. Increasing amounts of DA · HCl (200, 500, 1000, 1500 and 2000 ng) and DHTIQ·HBr (1, 10, 50, 100 and 1000 ng) were added. The samples were mixed (Vortex) and further treated as described below.

Possible artifactual formation of 3,3,4,4-tetradeutero DHTIQ (D_4DHTIQ) and DHTIQ identification. Individual rat brains (N = 5) were weighed and treated separately as described below. Each brain was homogenized in 2.5 ml of 14% $HClO_4$ containing 4000 ng of DDA · HCl.

Quantification of DA and DHTIQ. Weighed rat brains (N = 9) were homogenized separately as described below in 2.5 ml of 14% $HClO_4$ containing 2000 ng of DDA · HCl and 2000 ng of D_2DHTIQ·HBr.

All samples were mixed thoroughly and brought to a final volume of 5.0 with glass distilled water. The samples were then centrifuged (desk-top clinical) for 30 min and the supernatant fraction was decanted. The pellet was washed and rehomogenized with an additional 2.0 ml of 14% $HClO_4$ and centrifuged for 30 min. The supernatant fractions were combined and extracted with 2.0 ml of 15% isooctane in hexane by shaking for 15 min (Burrell 75 Wrist-Action Shaker). The organic phase was removed with a pasteur pipette and 0.25 ml of a solution containing 0.2% Na_2 EDTA and 6% thioglycolic acid was added to the aqueous phase. Phosphate buffer (3.0 ml, 0.01 M, pH 7.2) was added followed by the addition of 1.0 ml of absolute ethanol. The samples were placed in an ice bath and the pH was adjusted cautiously to 6.7 with 25% KOH and centrifuged to remove precipatated $KClO_4$. The supernatant fraction was passed through a 5.0 cm by 0.5 cm column of Amberlite CG-50 that had been washed with 10 ml of 0.2 M, pH 6.1, phosphate buffer. The eluent was discarded and the column was washed with 5.0 ml of ethanol/water (3.5, v/v), followed by 5.0 ml of glass distilled water. These were discarded and the column was washed with 5.0 ml of 0.4 N acetic acid containing 9%NaCl. The eluent was collected and treated with 1.0 g of $NaHCO_3$. Freshly distilled propionic anhydride (0.5 ml) was then added and the tri-phasic mixture was vortexed intermittently until all bubbling had ceased (approximately 1 hr). The reaction mixture was then extracted with two 2.0 ml portions of CH_2Cl_2. The extracts were combined, dried over Na_2SO_4 and evaporated to dryness under a stream of dry, high purity N_2. The residue was redissolved in 50 μl of CH_2Cl_2 and submitted for GC/MS analysis.

GC/MS analyses

The GC/MS analyses were conducted using a Hewlett Packard 5985A GC/MS equipped with a data analysis system. Gas chromatography was conducted on a Supelco 4 ft by 2 mm internal diameter glass column containing 2% SP-2250 on 100–120 mesh Chromosorb-W-HP. The column was maintained at 260° isothermal. High purity helium was used as the carrier gas at a flow rate of 40.0 ml/min.

Reference standards of the triproprionyl derivatives of DA, DDA, DHTIQ and D_2DHTIQ were prepared by reaction of the acid salts of the corresponding compounds with propionic anhydride as described (see "Preparation of samples for GC/MS analyses").

Electron impact (70 eV) mass spectra of each compound were characterized with respect to their base peak (normalized to 100 per cent) and other prominent secondary mass fragments (*m/z*). The retention times were recorded and the chosen diagnostic mass fragments were monitored for each compound in the selected ion monitoring (SIM) mode. Ion (*m/z*) intensity ratios were noted, and the per cent interference between the ions chosen for the proteo and deutero compounds was determined. Reference standards were examined in the SIM mode, recording retention times and ion intensity ratios, prior to and following analyses of the samples obtained from the tissue extracts to assure the stability of the instrumental conditions.

Three microliters of each derivatized brain sample was used for the analysis. Ions for the corresponding proteo and deutero compounds were monitored in the SIM mode. Following the analyses, the ion intensity ratios were calculated and used to identify and quantify endogenous DA and DHTIQ.

RESULTS

GC/MS analyses of standards

A representative chromatogram of the GC/MS analysis of the derivatized DDA and D_2DHTIQ standards is presented in Fig. 1. The electron impact mass spectra of derivatized DA, DDA, DHTIQ and D_2DHTIQ are shown in Fig. 2.(A–D, respectively). The ions (*m/z*) 192.0 and 136.0 were chosen to monitor for endogenous DA (Fig. 2A), whereas the ions (*m/z*) 195.0 and 140.0 were used to monitor the added DDA internal standard (Fig. 2B). The interference of the DDA ions to the DA ions was observed to be less than 0.1 per cent (Fig. 1, 99.9 per cent isotopic

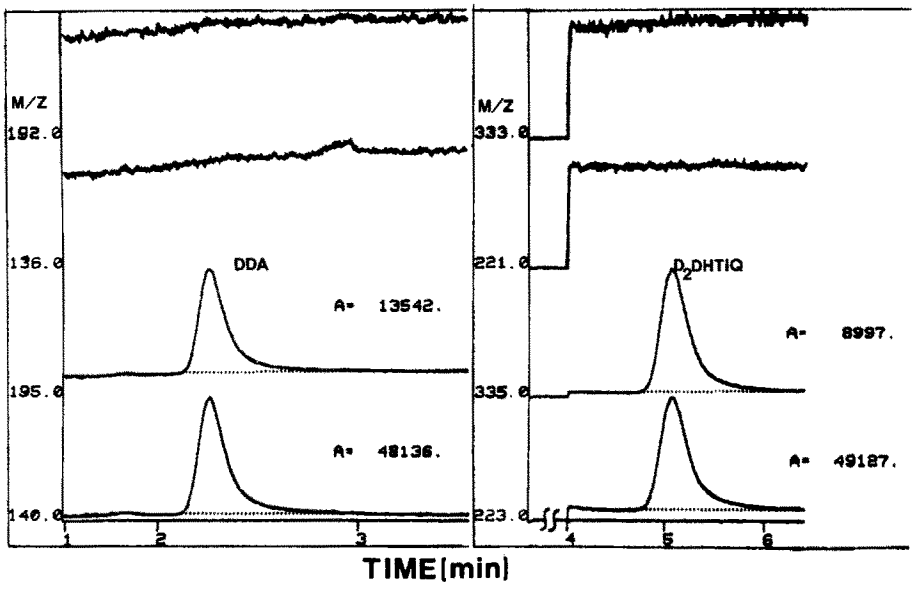

Fig. 1. Representative chromatogram of the gas chromatographic/mass spectrometric analysis of the tripropionyl derivatives of the DDA and D_2DHTIQ internal standards using selected ion monitoring (A = area).

purity). The ions (*m/z*) 333.0 and 221.0 were chosen to monitor for endogenous DHTIQ (Fig. 2C), whereas the ions (*m/z*) 335.0 and 223.0 were used to monitor for the added D_2DHTIQ internal standard (Fig. 2D). The interference of the D_2DHTIQ ions to the DHTIQ ions was also calculated to be less than 0.1 per cent (Fig. 1, 99.9 per cent isotopic purity). The ions 337.0 and 225.0 were used to monitor for the possible artifactual formation of 3,3,4,4-tetradeutero-DHTIQ (D_4DHTIQ) from endogenous HCHO and added DDA in the artifact formation and DHTIQ identification experiments.

As can be seen in panels A and B of Fig. 2 the 192.0 and 195.0 ions (*m/z*) for the respective proteo and deutero tripropionyl-DA standards resulted from the loss of one propionyl group (as $CH_3CH{=}C{=}O$) and cleavage of the C–N bond, accompanied by a hydride (2A) or deuteride (2B) transfer, to form the 4-propionyl-3-hydroxy-styrenyl charged species. There were, apparently, two competing pathways for the fragmentation of these compounds to give the 136 ion (*m/z*, 2A) and the 139 and 140 ions (*m/z*, 2B). The two possible structures are shown in Fig. 2A and 2B. Since there was an unequal distribution of the corresponding ions (136 and 139, 140) between the proteo and deutero compounds, the ions 192 and 195 were used for the quantification of DA. A plot of these ions for the standard curve yielded a slope of 0.98 and thus allowed for direct comparison of ion ratios for the endogenous DA and the added DDA internal standard.

The fragmentation of DHTIQ and D_2DHTIQ occurred with the loss of two propionyls ($CH_3CH{=}C{=}O$) from the molecular ions (*m/z* 333 and 335) to give the charged 3,4-dihydroxy-*N*-propionyl-TIQs (Fig. 2, panels C and D). The TIQ ring system in these compounds appeared to be quite stable, with no evidence of ring cleavage being observed in their mass spectra.

A more detailed exploration of the rather interesting fragmentation patterns of these compounds was beyond the scope of the present report. The formation of the two structures postulated for the 136 and 139 and 140 ions for DA and DDA has been confirmed by analysis of the tri-pentadeuteropropionyl DA derivatives and will be the subject of a forthcoming communication (Barker *et al.*, manuscript in preparation).

Linearity of recovery and limits of detection of DA and DHTIQ

GC/MS analyses of pooled rat brain samples containing a constant amount of added DDA·HCl and D_2DHTIQ·HBr (2000 ng of each) and increasing quantities of the corresponding proteo compounds showed that the recovery and detection of DA (*m/z* 192/195, slope = 0.98) and DHTIQ (*m/z* 333/335, slope = 0.97) were linear over a wide range of concentrations (1.0 ng to 2.0 μg). The limit of detection for DA was calculated to be approximately 1 ng/g wet weight of brain tissue, while that of DHTIQ was calculated to be approximately 2 ng/g.

Artifactual formation of D_4DHTIQ and DHTIQ identification

A representative chromatogram of the GC/MS analyses of five rat brain samples for endogenous DHTIQ and the possible artifactual formation of D_4DHTIQ from added DDA is presented in Fig. 3. The overall results are shown in Table 1.

The peaks for the ions monitored for endogenous DHTIQ were observed to have the same retention times and ion intensity ratios as those observed for the DHTIQ standards (Fig. 3; Table 1). We also observed the apparent artifactual formation of D_4DHTIQ. In many cases, however, the areas for the 337.0 ion were very near the limits of detection (Fig. 3). The ratio of the DHTIQ to D_4DHTIQ was 6.25:1 (Table 1).

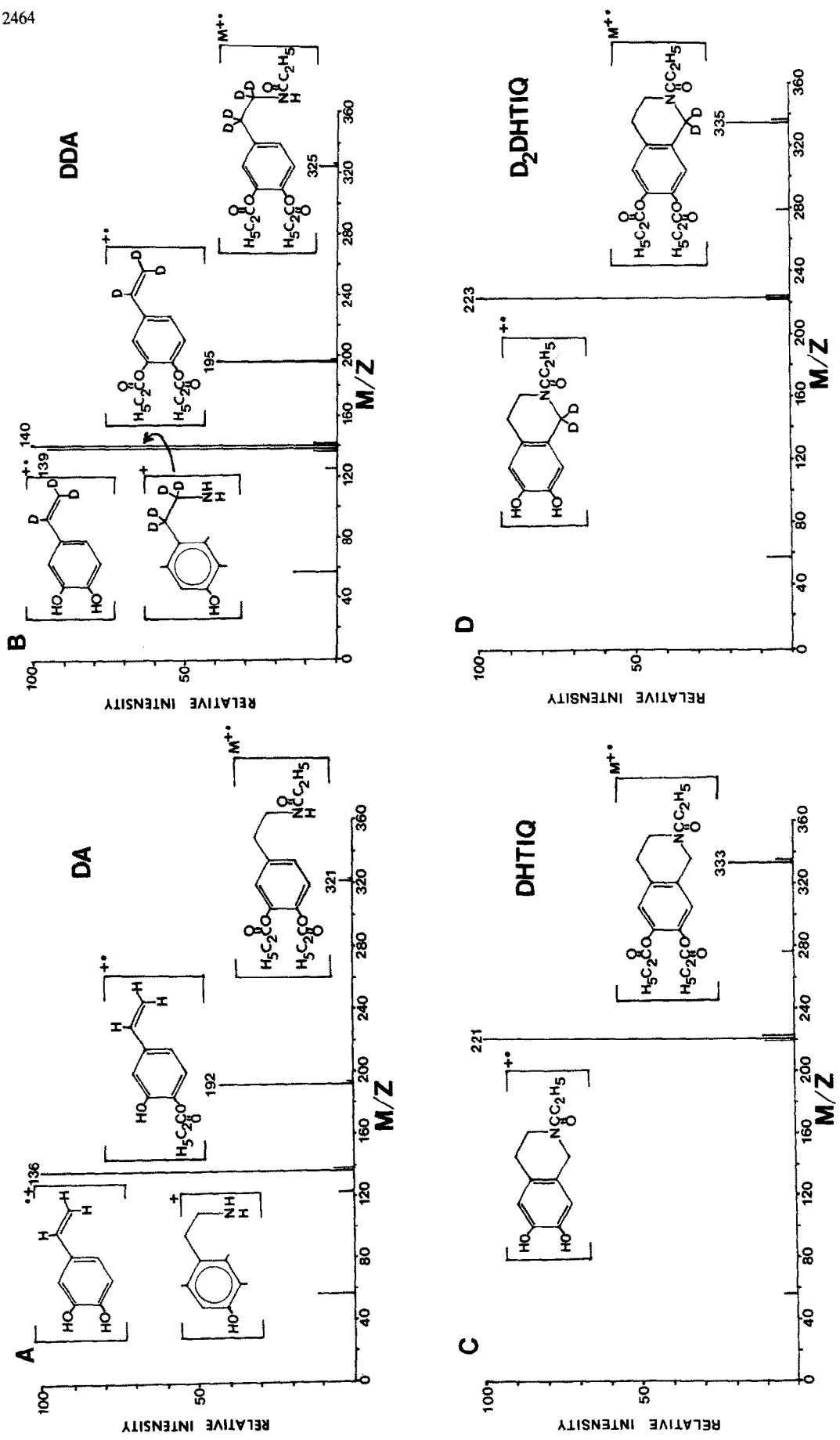

Fig. 2. The electron impact (70 eV) mass spectra of derivatized (A) DA, (B) DDA, (C) DHTIQ, and (D) D_2DHTIQ.

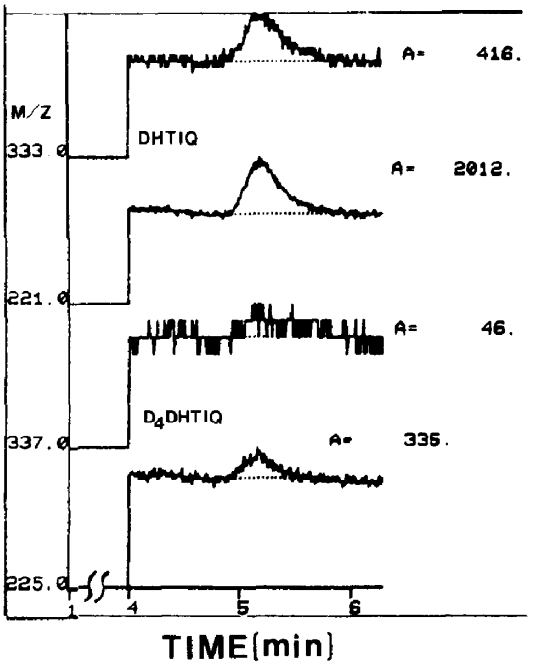

Fig. 3. Representative chromatogram for the gas chromatographic/mass spectrometric analysis of rat brain samples for endogenous DHTIQ (*m/z* 333.0 and 221.0) and the possible artifactual formation of D_4DHTIQ (*m/z* 337.0 and 225.0) from added DDA (A = area).

Quantification of DA and DHTIQ

By comparing the ratios of the ion (*m/z*) intensities for 192.0 (DA) and 195.0 (DDA), the level of DA in rat whole brain was determined to be 1.22 ± 0.22 μg/g wet weight (N = 14) (Tables 1 and 2; Fig. 4). Using the ions 333.0 for DHTIQ and 335.0 for D_2DHTIQ, the level of DHTIQ in rat brain was calculated to be 10.0 ± 3.0 ng/g wet weight (N = 9) (Fig. 4; Table 2). The ratio of nmoles DHTIQ to nmoles DA in these nine rat brain samples was 0.0083 ± 0.0026 or 0.83 ± 0.26 per cent (Table 2). The value obtained for the whole brain DA (1.22 ± 0.22 μg/g) is in good agreement with values reported by other (gas chromatographic and fluorescence) techniques [14–16].

DISCUSSION

The fact that GC/MS peaks derived from the tissue samples were observed for the ions monitored for DHTIQ at the correct retention times and that the areas of these peaks had the same ion intensity ratio as that observed for the DHTIQ standard may be taken as evidence for the identification of DHTIQ as a normal constituent of rat brain. However, we also observed the apparent artifactual formation of D_4DHTIQ from added DDA (Fig. 3). Thus, the question of whether or not the DHTIQ identified in this study was formed merely as an artifact of the procedure must be asked. The ratio of endogenous DA to added DDA was 0.87 ± 0.14 (Table 1). Therefore, from the point preceding homogenization, DA and DDA were present in nearly equal concentrations. In most cases the level of added DDA was higher than the level of endogenous DA. This being the case, the ratio of DHTIQ to D_4DHTIQ was calculated to be 6.25:1. Thus, the amount of D_4DHTIQ formed represented 16 ± 4 (±S.D.) per cent of the amount of DHTIQ observed. A 1:1 ratio of these compounds would be expected if their formation was totally artifactual since their precursors were present in near equal amounts. Thus, approximately 84 ± 6 per cent of the DHTIQ identified in this study appears to have been formed *in vivo*, apparently derived from the *in vivo* condensation of dopamine with formaldehyde [17–21].

β-Carbolines and TIQs have been identified as natural products of a variety of hallucinogen-containing plants. Several β-carbolines have now been identified as normal constituents of mammalian brain, adrenal gland, blood and urine [4–10]. Furthermore, the identification of several types of TIQs formed *in vivo* following ethanol or L-dihydroxyphenylalanine (L-DOPA) administration has now been reported [22–26]. Collins *et al.* [22] have recently identified the *o*-methylated derivatives of the dopamine-acetaldehyde condensation product, salsolinol, as normal components of human urine (20.6 ± 7.1 μg/24 hr) (±S.D.). The present study demonstrates the occurrence of DHTIQ as a normal constituent of brain tissue, not requiring any pharmacological intervention or precursor loading for its formation and subsequent detection.

Table 1. Results of artifactual formation of D_4DHTIQ and DHTIQ identification experiments*

Sample	Brain wt (g)	Ratio of endogenous DA:added DDA (*m/z*, 192/195) (4000 ng DA·HCl)	DA ion ratio (*m/z*, 192/136)	DA RT (min)	DA (μg/g) (*m/z*, 192/195)	DHTIQ ion ratio (*m/z*, 333/221	DHTIQ RT (min)	D_4DHTIQ/DHTIQ (*m/z*, 225/221)
Standards			0.23 ± 0.006 (N = 5)	2.1		0.17 ± 0.02 (N = 5)	4.8	
1	2.09	0.64	0.23	2.1	0.989	0.16	4.8	0.22
2	2.12	0.90	0.23	2.1	1.374	0.20	4.8	0.09
3	1.88	1.00	0.24	2.1	1.722	0.17	4.8	0.22
4	2.05	0.96	0.23	2.1	1.512	0.21	4.8	0.17
5	2.08	0.84	0.24	2.1	1.302	0.18	4.8	0.10
Average	2.04 ± 0.10	0.87 ± 0.14	0.23 ± 0.005	2.1	1.38 ± 0.271	0.18 ± 0.02	4.8	0.16 ± 0.06

* Each result is the average of N values ±S.D. RT = retention time.

Table 2. Results of the gas chromatographic/mass spectrometric analysis of rat brain samples for the quantification of DA and DHTIQ*

Sample	Brain wt (g)	DA/DDA (m/z, 192/195) (2000 ng DDA·HCl	DA ion ratio (m/z, 192/136)	DA RT (min)	DA (μg/g) (m/z, 192/195)	DHTIQ/ D_2DHTIQ (m/z, 333/335) (2000 ng D_2DHTIQ·HBr)	DHTIQ ion ratio (m/z, 333/221)	DHTIQ RT (min)	DHTIQ (μg/g) (m/z, 333/335)	nmoles DHTIQ/ nmoles DA
Standards			0.23 ± 0.006 (N = 5)	2.1			0.17 ± 0.02 (N = 5)	4.8		
6	1.92	1.26	0.23	2.1	1.060	0.023	0.15	4.8	0.015	0.013
7	2.08	1.52	0.24	2.1	1.183	0.025	0.21	4.8	0.016	0.012
8	2.00	1.27	0.23	2.1	1.026	0.012	0.18	4.8	0.008	0.007
9	2.08	1.63	0.23	2.1	1.269	0.018	0.15	4.8	0.011	0.008
10	2.04	1.39	0.23	2.1	1.101	0.013	0.20	4.8	0.009	0.008
11	2.04	1.17	0.23	2.1	0.926	0.012	0.19	4.8	0.008	0.008
12	1.83	1.40	0.23	2.1	1.235	0.014	0.14	4.8	0.010	0.008
13	2.28	1.88	0.22	2.1	1.335	0.014	0.16	4.8	0.008	0.005
14	1.98	1.27	0.23	2.1	1.038	0.011	0.18	4.8	0.007	0.006
Average	2.03 ± 0.12	1.42 ± 0.22	0.23 ± 0.005	2.1	1.130 ± 0.133	0.016 ± 0.005	0.17 ± 0.02	4.8	0.010 ± 0.003	0.0083 ± 0.0026

* Each result is the average of N values ±S.D. RT = retention time.

Comparison of DHTIQ brain levels to those of DA and THBCs

The formation of DHTIQ by treating tissue samples with gaseous HCHO has been used as a method for the fluorescence analysis of DA for many years [27]. Thus, the relatively low level of DHTIQ reported here is not surprising, since much higher levels could have significantly affected tissue blanks in the fluorescence assay of DA. What is interesting, however, is a comparison of the levels of DHTIQ and of the β-carbolines, THBC and 6-methoxy-THBC (6-MeOTHBC), reported in rat whole brain.

The apparent precursor in the Pictet–Spengler formation of THBC *in vivo* is tryptamine (TA), which has been measured in rat whole brain as less than 500 pg/g wet weight [28]. The amount of THBC in rat whole brain, however, has been reported as 17.5 ng/g [9] and in rat forebrain as 47.3 ng/g [5]. The precursor for the formation of 6-MeOTHBC, 5-methoxy-TA (5-MeOTA), has been reported in rat brain as 2.2 ng/g [29], whereas the brain level of 6-MeOTHBC has been reported as 35.6 ng/g [9]. Although the level of DA is many times greater than the level of TA or 5-MeOTA in whole brain, the amount of DHTIQ is less than that of THBC or 6-MeOTHBC. Since the relative rates of reaction of DA and TA with HCHO are approximately 100:1 [11, 30], and the relative concentration of DA to TA and 5-MeOTA is so much greater, one could speculate that THBC formation may occur by enzymatic catalysis. DHTIQ formation may occur solely by non-enzymatic mechanisms. However, DA may be protected from reaction with HCHO by compartmentalization of vesicular storage, or the turnover rate of the DHTIQ may be much greater than that for the THBCs. Thus, until the levels of the DHTIQ, THBCs and their metabolites can be measured in the same animals and further studies are conducted in brain regionalization of these compounds and their possible enzymatic formation, the concept of specific biosynthetic mechanisms for THBCs must remain hypothetical.

Pharmacological significance of DHTIQ

Compared to the whole brain levels of other DA metabolites [31] the brain level of DHTIQ might well be considered as being minor. Circumstances may arise, however, where the level of DHTIQ could be elevated to the point at which it could exert significant pharmacological effects. For example, Riggin and Kissinger [23] have recently reported the identification of DHTIQ in the urine of rats administered 100 mg/kg of L-DOPA. Thus, the level of DHTIQ may be elevated *in vivo* during therapeutic treatments that elevate brain dopamine (monoamineoxidase inhibition, L-DOPA therapy for Parkinsonism,etc.). The brain level of DHTIQ may also be elevated by inhibition of aldehyde, particularly HCHO, metabolism. This may occur in deficiencies of co-factors for aldehyde metabolizing enzymes (nicotinamide, folic acid, glutathione, etc.) or in the adminstration of aldehyde metabolism inhibitors (disulfiram, etc.). Thus, the levels of DHTIQ may be of particular interest in alcoholism where, especially during withdrawal, the levels of methanol, HCHO and formate are elevated significantly [32–

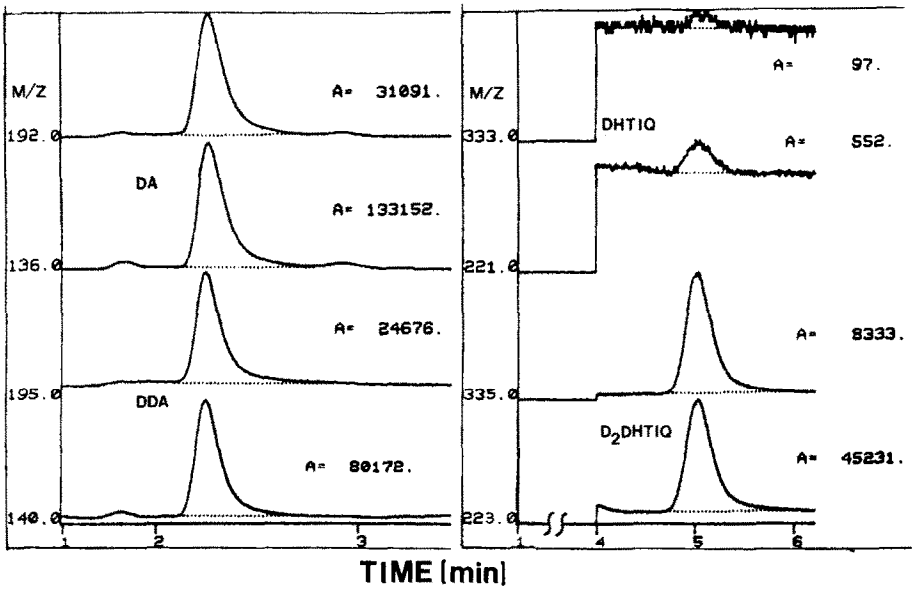

Fig. 4. Representative chromatogram of the gas chromatographic/mass spectrometric analysis of rat brain samples for the quantification of DA and DHTIQ (A = area).

35] and the co-factors nicotinamide, folic acid and glutathione are often depleted [36]. Although measurable quantities of the acetaldehyde-DA condensation product, salsolinol, have now been identified in man and other mammals following alcohol administration [22, 25], there has yet to be an attempt to measure the levels of the HCHO-DA product that may result from alcohol abuse. Formaldehyde is fifteen times more reactive than acetaldehyde in the Pictet–Spengler reaction with dopamine [11]. Furthermore, the condensation products formed with HCHO do not introduce a chiral center as does acetaldehyde. Thus, the condensation products of HCHO and biogenic amines should also be considered in studies of alkaloid formation resulting from alcohol abuse. Formaldehyde-derived TIQs and THBCs have both been shown to increase alcohol consumption in the rat, an effect that does not appear to abate after their administration ceases (see Ref. 1). The fact that such compounds occur normally in brain tissues provides an interesting area for further inquiry.

Recent experiments in this laboratory have detected what appears to be abnormally high HCHO concentrations in the urine of some schizophrenic patients [37]. If these preliminary studies are substantiated in a large patient population, then one might speculate that high levels of HCHO in the CNS could lead to increased levels of TIQs and THBCs, especially DHTIQ, and that an increase in these compounds could be responsible for certain aspects of the symptomatology of this disease [38]. TIQs have been shown to act as "false transmitters", being taken up, stored, and released by mechanisms similar to those of other catecholamines (for a review see Refs. 1–3). These compounds also inhibit the uptake of catecholamines, stimulate catecholamine release, and inhibit monoamine oxidase, catechol-*o*-methyltransferase, as well as dopamine-sensitive adenylate cyclase activities (see Refs. 1–3). Although the brain level of DHTIQ reported here is quite low in comparison to that of other biogenic amines in the brain, it may, under certain circumstances, exert an important effect on brain function and, thus, behavior. This could be true especially if certain brain areas are more subject to exposure to endogenously produced HCHO than are others.

The relative concentrations of DHTIQ and the THBCs thus far identified in rat brain appear to indicate that THBC formation may occur by enzymatic catalysis. However, until further experiments are conducted as described, this possibility must remain a matter for speculation and further inquiry. It is becoming more evident, however, that TIQ and THBC formation does normally occur *in vivo* and may play an as yet undetermined role in normal as well as abnormal brain function.

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