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Analysis of 5-methoxytryptamine at the femtomole level in the rat and quail brain by gas chromatography–electron-capture negative-ion chemical ionization mass spectrometry

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Abstract

A sensitive method for the measurement of endogenous 5-methoxytryptamine in brain tissue has been developed using capillary column gas chromatography–electron-capture negative-ion chemical ionization mass spectrometry. 5-Methoxytryptamine was first converted to N-[$^2H_3$]acetyl-5-methoxytryptamine by reaction with hexa-deuterated acetic anhydride, followed by reaction with pentafluoropropionic anhydride to yield the highly electron-capturing 3,3′-spirocyclic pentafluoropropionyl indolenine derivative. Quantitative analysis was carried out by selected-ion monitoring of the $[M-HF]^-$ and $[M-HF-DF]^-$ ion intensity of the 3,3′-spirocyclic pentafluoropropionyl indolenine derivative, using 5-methoxy-[$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine as the internal standard. The presence of 5-methoxytryptamine in the brain tissue was demonstrated. In the absence of a monoamine oxidase inhibitor, the mean±S.D. levels of 5-methoxytryptamine in the rat and quail whole brain were found to be 30±6 and 347±52 pg/g, respectively. The possible physiological functions of 5-methoxytryptamine as a neuromodulator and/or neurotransmitter have to be considered.

Keywords: 5-Methoxytryptamine

1. Introduction

5-Methoxytryptamine (5MT) is a physiologically active methoxyindole derived from 5-hydroxytryptamine (serotonin), a well recognised neurotransmitter of the brain. Abnormal amount of 5MT in the central nervous system could produce LSD-like disruptive effects on behaviour [1], and elevated levels of 5MT were reported in manic and acute schizophrenic patients [2]. Moreover, 5MT was also found to be a potential precursor in the biosynthesis of the potent psychotomimetic agent 5-methoxy-N,N-dimethyltryptamine [3] and of the putative neuromodulators, the β-carbolines [4].

Injection of 5MT also induced physiological and behavioural changes such as hindlimb scratching in the rat [5], tachycardia in pigs [6], vagus nerve depolarization in rats [7], sleep promotion in rodents [8], vomiting in dogs [9], suppression of guinea pig ileum contraction [10], and inhibition of reproductive functions in golden hamsters [11].

The pharmacological and/or physiological actions

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of 5MT reported in mammals are considered by many to be mediated by a serotonin receptor subtype, 5-HT1D (5-HT4) [5–7,10,12]. In fact, 5MT is a specific agonist of 5-HT1D receptors and is used as a pharmacological tool for 5-HT1D receptor identification [5–7,10,12,13]. 5-HT1D receptors have been cloned and sequenced [13]. The pharmacological properties of human brain 5-HT1D receptors have been characterized [14]. The physiological effects and functions of 5MT have been reviewed by Pévet et al. [15,16].

In the biological tissues studied so far, methoxyindoles are present in much lower amounts (low nanogram to picogram levels) than hydroxyindoles. The presence of 5MT has been reported in the pineal gland [17–19], brain tissues [20–22] and plasma of mammals [23,24]. Analytical techniques such as UV-Vis fluorescence spectrophotometry [20,22], high-performance liquid chromatography [24–26] and gas chromatography–mass spectrometry (GC–MS) [17,26–28] have been employed to determine the endogenous levels of 5MT in biological samples. However, there are only scanty reports on the endogenous 5MT levels in the brain tissues of laboratory animals, with a large discrepancy among the levels reported [29]. Furthermore, these findings are regarded as inconclusive due to insufficient specificity and sensitivity of the analytical method used, as well as artifacts arising from the derivatization reaction in GC–MS analysis [16,30]. An additional problem is that 5MT is likely to be rapidly catabolized by monoamine oxidase (MAO) [31], an enzyme also found in the brain [32]. In many of the recent physiological studies conducted, 5MT levels were successfully measured only after the laboratory animals were pre-treated with a monoamine oxidase inhibitor [11,26].

In an attempt to solve this problem, we report here the development of an ultra sensitive analytical method based on GC–electron-capture negative-ion chemical ionization MS–selected ion monitoring (GC–EC-NICI-MS–SIM) capable of measuring the femtomole levels of endogenous 5MT in the whole brain of rat and quail. This technique has been applied to the measurement of other indolealkylamines [28,33,34] and common biogenic amines [35], but 5MT was not detected in the bovine eye and human plasma [28,35].

2. Experimental

2.1. Reagents

Dichloromethane, ethyl acetate, methanol and isooctane (reagent grade, Merck, Darmstadt, Germany) were doubly distilled before use and stored in reagent bottles over molecular sieves. 5MT, N-acetyl-5-methoxytryptamine (Ac-5MT, melatonin) and 5-methoxy-[$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine hydrochloride (d_4-5MT, >99% purity) were purchased from Sigma (St. Louis, MO, USA). d_6-Acetic anhydride (99 atom-% D) was purchased from MSD Isotopes (Dorval, Canada). O-Methyl-[$^3H_3$]melatonin (70–85 Ci/mmol) was obtained from Amersham International (Buckinghamshire, UK). Pentafluoropropionic anhydride was purchased from Aldrich (Milwaukee, WI, USA). Other chemicals were all analytical grade, and unless otherwise specified, were used without further purification.

2.2. Sample preparation, extraction and derivatization

Male Sprague–Dawley rats (age 6–7 week, average body weight 191±17 g, mean±S.D., n=24) were obtained from the Laboratory Animal Unit, University of Hong Kong. Male quails (average body weight 108±18 g, mean±S.D., n=6) were obtained in one batch from a local market in Hong Kong. The rats and quails were adapted for at least 7 days to a 12–12 h light–dark cycle prior to sacrifice. They were decapitated at mid-light, and whole brains were removed as quickly as possible, freed from other tissues, weighed, and individually put inside polyethylene centrifuge tubes each containing 6 ml of perchloric acid (0.4 M) and a known amount (~1–0.1 ng) of d_4-5MT as the internal standard. The tissue samples were then homogenized with a Polytron homogenizer inside an ice-bath, followed by centrifugation at 14 000 g for 30 min to remove the precipitated proteins, and the supernatants were decanted to silanized culture tubes. The time taken from dissection to homogenization of brain tissue samples was less than 3 min.

The pH of the aqueous supernatant was adjusted to 1.5 by dropwise addition of potassium hydroxide solution (4.0 M). Fatty substances were removed by

repeated extractions with petroleum ether (5 ml, b.p. 60–80°C). The fat content was found to vary with individual samples and 4–6 times of extraction were necessary for brain samples. The removal of fatty substances must be done thoroughly; it was found to be a pre-requisite to a "clean" GC–MS–SIM chromatogram. Endogenous melatonin in the sample was removed by extraction with dichloromethane (3×6 ml). The pH of the aqueous fraction was adjusted to 12.5, and 5MT was extracted into dichloromethane (2×6 ml). The final dichloromethane extracts were pooled ($n=1$ for quail samples, $n=4$ for rat brain samples), transferred to a silanized glass reacti-vial and dried under a gentle stream of nitrogen.

The efficiency of the extraction procedure was assessed by spiking 5MT standard solutions (500 pg/ml) and brain homogenates with radioactive [$^3H_3$]5MT prepared in our laboratory (specific activity, ~60–85 Ci/mmol) (Tsang, unpublished results) and the radioactivities were measured before and after the extraction steps. The % recoveries were found to be 75±4% and 78±4% (mean±C.V., $n=6$) for 5MT standard solutions and quail brain samples respectively, which were comparable to the % recoveries reported earlier by Raynaud and Pévet using slightly different extraction procedures [25]. Similar studies using N-acetyl-5-[$^3H_3$]methoxytryptamine (*O*-methyl-[$^3H_3$]melatonin) showed that 96±4% (mean±C.V., $n=6$) of endogenous melatonin in the sample was removed by dichloromethane at pH 1.5.

d_6-Acetic anhydride (25 μl) was added to the dried extracts, vortex-mixed for 1 min, and then heated at 60°C for 15 min. Excess reagent was removed under a gentle stream of nitrogen followed by vacuum drying at 40°C for 30 min. The residue was redissolved in ethyl acetate (50 μl), pentafluoropropionic anhydride (50 μl) was added, vortex-mixed for 30 s, and finally heated at 60°C for 45 min. After cooling, excess reagent and solvent were removed under a gentle stream of nitrogen, followed by vacuum drying at 40°C. The derivative was stored at −20°C and analysed by GC–MS the next day.

For GC–MS analysis, the dried derivatized sample was re-dissolved in 50–100 μl of iso-octane, reduced to approximately 10 μl by gentle blowing under nitrogen, and 2-μl aliquots were injected directly onto the GC column.

In this study, the brain samples were homogenised in perchloric acid immediately after they were taken from the animal body to minimize possible sample loss due to enzymatic degradation and sample storage, followed by immediate sample extraction and derivatization, which were carried out with efficiency and on the same day. Special care was taken to protect the samples from bright light during the homogenization, extraction and derivatization steps. We have found that these precautionary measures were necessary to obtain reproducible quantitative results.

2.3. GC–MS

The GC–MS system used in this work consisted of a Pye 204 gas chromatograph directly coupled to a VG 7070F magnetic sector mass spectrometer, and operated under the control of the VG 2350 data system comprised of a Mark II digital scanner (digital control of accelerating voltage and magnetic field), a Mark II data acquisition interface (signal digitization and communication with control computer) and a PDP-11/23 (host) and PDP-8A (control) computer.

A Hewlett-Packard cool on-column injector was installed in the PYE 204 gas chromatograph, replacing the original split–splitless injector for total injection of sample at temperature 40–50°C below the boiling point of iso-octane, the solvent chosen for sample injection. The GC conditions were: 30 m× 0.32 mm I.D., 0.17 μm OV-101 film thickness fused-silica capillary column (Hewlett-Packard, Ultra-1); helium carrier gas flow controlled by constant column head pressure with a linear velocity of 50–60 cm/s at 220°C (as indicated by the elution time for pentane); GC–MS direct interface at 250°C; initial column temperature at 40°C rapidly heated to 220°C for 20 min. Sample introduction (2 μl) was by direct injection through the cool on-column injector at room temperature with a 11 cm×0.17 mm O.D. fused-silica syringe needle onto the GC capillary column.

The VG 7070F magnetic sector mass spectrometer was originally equipped with a Cu/Be electron multiplier for positive-ion detection only. To overcome this problem, a negative-ion detection system based on a home-built isolation amplifier modified from the design first reported by Smit and Field [36]

was constructed and installed. In the EC-NICI mode of operation, the first dynode of the multiplier was held at ground potential and the last dynode was held at +1.5 to +2.5 kV to produce secondary electrons in good yield. The output signal (referenced at 1.5 to 2.5 kV above ground potential) was conducted by a floating coaxial feedthrough first to a preamplifier (Analog Device 545 operational amplifier) and then to an isolation amplifier (Analog Device 277J) which transformed and outputed the signal at ground potential for further amplification by the fast-response amplifier of the MS system [37]. With the isolation amplifier installed, we were able to detect 0.15 pg (nominal)/injection of the 3,3′-spirocyclic pentafluoropropionyl indolenine derivative of melatonin at a signal-to-noise (S/N) level of 2.5, a detection limit which was more than sufficient for this study. This simple and low-cost negative-ion detection system has been operating satisfactorily on our VG 7070F mass spectrometer for the past two years and is a practical solution in existing magnetic-sector mass spectrometers originally not designed and equipped with a negative-ion detection system.

The EC-NICI–MS conditions were: accelerating voltage 4 kV; electron energy 50/100 eV; electron emission current 2000 μA; ion source temperature 200–220°C and reagent gas (methane) pressure at $(3-7)\cdot 10^{-5}$ mbar (as measured with an ionization gauge underneath the ion source). The sensitivity in the EC-NICI mode was optimized by tuning the ion intensities of the mass scale calibration compound, high b.p. perfluorokerosene. To increase the ion-collection efficiency and sensitivity in the EC-NICI mode, the ion-exit slit of the ion source was enlarged to 2.0×2.5 mm.

2.4. Quantitative analysis

Under the experimental and GC–MS conditions employed in this study, only one major peak with a retention time of 5.5 min was observed in the GC–MS total ion current chromatogram. SIM was carried out by accelerating voltage switching at mass resolution ($M/\Delta M$) of 600–800, ion dwelling time of 200 ms and channel switching time of 100 ms. Instrumental control and subsequent data processing of SIM runs were carried out with the help of the VG FBSIR software package.

To obtain a standard calibration curve, a set of standard solutions containing different amounts of 5MT (0.1–1.0 ng) and the same amount of d_4-5MT (1 ng) internal standard was prepared by serial dilution of stock solutions, dried under a gentle stream of nitrogen, and derivatized in the same way as the brain samples described earlier.

The ion currents monitored were m/z 321/324 and m/z 342/345 for the 3,3′-spirocyclic pentafluoropropionyl indolenine derivative of N-[$^2H_3$]acetyl-5-methoxytryptamine (d_3-Ac-5MT) and its deuterated internal standard N-[$^2H_3$]acetyl-5-methoxy-[$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine ($d_3$-Ac-$d_4$-5MT). In addition, the ion intensities at m/z 320 and m/z 340 due to melatonin were also monitored. In the sample mass chromatograms, they were either not observed or relatively low compared to the m/z 321/324 and m/z 342/345 ion intensities, indicating the absence or near absence of melatonin in the brain extracts in this study. For other biological samples which contain a high concentration of melatonin relative to 5MT, the m/z 320/340 ion intensities may be comparable to the m/z 321/342 ion intensities. In such cases, the ion intensity at m/z 321 has to be corrected for the ^{13}C contribution from the peak at m/z 320 due to endogenous melatonin.

Quantitative analysis was performed by comparing chromatographic peak-area ratios (sample/internal standard) of sample against a standard calibration curve which was obtained by plotting peak-area ratios (5MT standard/d_4-5MT internal standard) against the relative amounts of 5MT and d_4-5MT internal standard. If the amount of 5MT found at these two m/z intensity ratios agreed within ±20%, then the presence of 5MT in the biological sample was accepted. The average of 5MT levels measured at m/z 321/324 and m/z 342/345 was taken as the final result.

3. Results and discussion

In this study, 5MT was first converted to d_3-Ac-5MT by reaction with hexa-deuterated acetic anhydride, followed by an intra-molecular cyclization reaction with pentafluoropropionic anhydride. Previous studies have shown that after injection into

a heated GC column, the derivatization product is a highly electron-capturing 3,3′-spirocyclic pentafluoropropionyl indolenine derivative, 1-pentafluoropropionyl - 2 - methylenepyrrolidine - 3 - spiro-3′-(3H-5-methoxyindole) [38,39], or its deuterated analogs adopted in this study (refer to Fig. 1). N-Acetylation by acetic anhydride was used in a similar study on tryptamine [34]. Due to the presence of melatonin in our biological samples [40], acetic anhydride was not adopted in this study because the derivatization product would be identical for 5MT and melatonin, which could lead to false high levels of 5MT in subsequent GC–MS–SIM measurements. Such a problem is eliminated by the use of d_6-acetic anhydride and initial formation of d_3-Ac-5MT in this study.

The EC-NICI mass spectra of the 3,3′-spirocyclic pentafluoropropionyl indolenine derivatives of melatonin, d_3-Ac-5MT and d_3-Ac-d_4-5MT are shown in Fig. 1. In agreement with the literature [33], the EC-NICI spectrum of the 3,3′-spirocyclic pentafluoropropionyl indolenine derivative of melatonin was dominated by the $[M-HF-HF]^{-}$ ($[M-2HF]^{-}$) ion (base peak, ~80% of total ionization current, Fig. 1a), with no molecular anion M^{-} and few additional fragment ions. Presumably, the $[M-HF-HF]^{-}$ ion (m/z 320) is formed by consecutive loss of 2 HF neutrals from the molecular anion, as indicated by the presence of a $[M-HF]^{-}$ ion (m/z 340, ~10% of base peak) in the spectrum. Corresponding $[M-HF]^{-}$ ion (m/z 342) (Fig. 1b, no HF elimination involving methylene hydrogens in the pyrrolidine ring) and $[M-DF]^{-}$ ion (m/z 345) (Fig. 1c, no HF elimination involving hydrogens on the indole skeleton) were observed in the spectra of the 3,3′-spirocyclic pentafluoropropionyl indolenine derivatives of d_3-Ac-5MT and d_3-Ac-d_4-5MT respectively. This suggests that the $[M-HF]^{-}$ ion is formed by specific elimination of one of the $\alpha,\alpha,\beta,\beta$-hydrogens from the 3-position ethylamine side-chain.

The very abundant and analyte-specific $[M-HF-HF]^{-}$ ion was suitably employed in GC–EC-NICI-MS–SIM analysis of melatonin in rat plasma [33]. Corresponding pairs of ions, $[M-HF-DF]^{-}$ ion (m/z 321)/$[M-HF-HF]^{-}$ (m/z 322) and $[M-DF-DF]^{-}$ (m/z 324)/$[M-DF-DF]^{-}$ (m/z 325), were observed in the EC-NICI mass spectra of the d_3-Ac-5MT and d_3-Ac-d_4-5MT derivatives respectively (Fig. 1b and c). The results indicated that the deuterated analogs of the $[M-HF-HF]^{-}$ fragment ion were formed by elimination of the second HF neutral involving the H/D atoms of (i) the 3-position ethylamine side chain, (ii) the methylene group on the pyrrolidine ring and (iii) the indole skeleton.

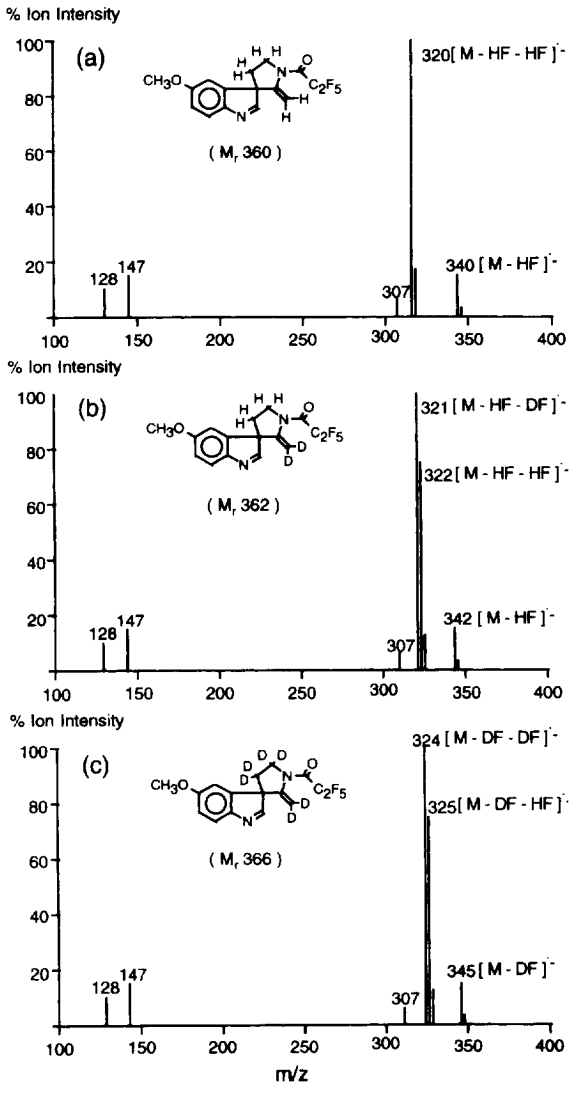


Fig. 1. EC-NICI mass spectra of 3,3′-spirocyclic pentafluoropropionyl indolenine derivative of (a) N-acetyl-5-methoxytryptamine (Ac-5MT, melatonin), (b) N-[$^2H_3$]acetyl-5-methoxytryptamine (d_3-Ac-5MT) and (c) N-[$^2H_3$]acetyl-5-methoxy-[$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine ($d_3$-Ac-$d_4$-5MT)

Based on the relative intensities of the m/z 321/322 (100:75) and m/z 324/325 (100:50) ion pairs, the contributions from these three sources of hydrogen/deuterium were estimated to be 24, 43 and 33%, respectively.

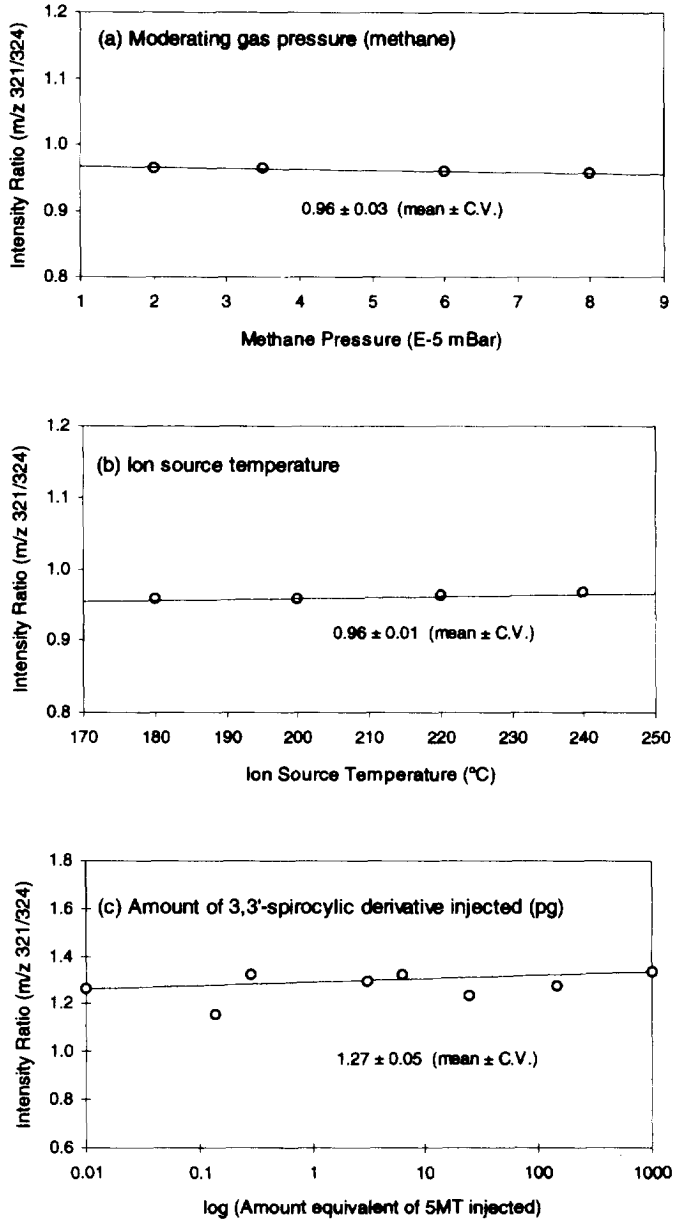
Due to the depletion of thermal electrons and varying amounts of neutral species within the ion source, the relative abundances of $[M-H]^-$ and $[M-HF]^-$ ions in the EC-NICI spectra of N-pentafluoropropionyl-*O*-*n*-butyl derivatives of amino acids were reported to be affected by GC–MS instrumental conditions and analyte concentrations [41]. To ensure that the relative $[M-HF-DF]^-/[M-DF-DF]^-$ ion intensities were unaffected by GC–MS conditions and possible H/D isotope effects, the m/z 321/324 intensity ratio was measured as a function of analyte concentration, moderating gas (methane) pressure and ion source temperatures. The results obtained for a standard solution containing 5MT and d_4-5MT (1:1) are shown in Fig. 2. For an ion source temperature range of 180–240°C and methane gas pressure (vacuum gauge reading) of $(2.0–8.0)\cdot10^{-5}$ mbar, the m/z 321/324 ion intensity ratio remained essentially constant with coefficient of variation equal to 1% and 3% respectively. This suggested that within the range of temperature and pressure tested, the temperature of ionization chamber and the pressure of moderating gas were not critical instrumental parameters affecting the ion intensity ratio. There was also no significant difference in the measured ion intensity ratio if different amounts of analyte derivatives equivalent to 0.1 to 150 pg of 5MT and d_4-5MT were injected, which was the analytical range encountered in actual sample analysis. The coefficient of variation was 5%. The absence of an analyte concentration effect is not surprising since at picogram levels of analyte injected, the thermal electron population within the ion source is far from depleted, and the anion/neutral concentrations are too low to produce significant ion–molecule reaction products.

As a result, the $[M-HF-DF]^-/[M-DF-DF]^-$ (m/z 321/324) intensity ratio derived from the 3,3′-spirocyclic pentafluoropropionyl indolenine derivatives of d_3-Ac-5MT and d_3-Ac-d_4-5MT was finally adopted for quantitative analysis of 5MT in biological samples. Similarly, the $[M-HF]^-/[M-DF]^-$ (m/z 342/345) intensity ratio was also used to provide confirmatory data, though the analytical sensitivity was reduced due to their low abundances.

Fig. 3 shows the m/z 321 ion intensity trace of the 3,3′-spirocyclic pentafluoropropionyl indolenine derivative of d_3-Ac-5MT in the GC–EC-NICI-MS–SIM chromatogram. The chromatographic peak was clearly visible when an equivalent of 0.5 pg (nominal) of 5MT standard was injected even when the gain factors of the amplifier and Cu/Be multiplier of the mass spectrometer system were not set at their most sensitive limits. The very good detection limit achieved showed that the home-made isolation amplifier negative-ion detection system is sufficient for the ultra-trace analysis of 5MT in brain tissues. No GC peak was observed in the chromatogram of the control blank. Typical GC–EC-NICI-MS–SIM chromatogram profiles of 5MT in quail and rat whole brain samples are shown in Fig. 4. These profiles showed very clear and distinct analyte peaks, though the background chemical interferences were more visible for rat brain samples which contained lower levels of 5MT.

The standard calibration curves for the high-mass (m/z 342/345) and low-mass (m/z 321/324) fragment ions both displayed linearity ($y=1.26x+0.08$ and $y=1.20x+0.09$, respectively) with a high correlation coefficient of 0.99. A sensitivity of 200 and 2000 area counts per picogram of analyte were obtained for the high-mass and low-mass ion respectively. Repeated analysis of 5MT/d_4-5MT standard solutions showed an intra-assay and inter-assay coefficient of variation of 3% and 9% respectively ($n=6$). The very good intra-assay and inter-assay reproducibility suggested that the analytical protocols developed were suitable for analysis of 5MT in biological samples.

The 5MT levels in brain samples measured by monitoring ion intensity ratios at m/z 321/324 were compared to that obtained at m/z 342/345. The ratios of 5MT levels obtained by these two pair of ions (m/z 321/324 versus m/z 342/345) were 0.94 ± 0.15 and 0.93 ± 0.14 (mean±S.D., $n=6$) for rat and quail brain samples respectively. The good consistency in 5MT levels measured at high mass (m/z 342/345) and low mass (m/z 321/324) intensity ratios demonstrated the presence of endogenous 5MT in the brain of rat and quail. The average levels of endogenous 5MT in rat and quail whole brain



* m/z 321/324 intensity ratio obtained for a derivatized 5MT/d_4-5MT standard mixture (1:1)

Fig. 2. Dependence of ion intensity ratio (*m/z* 321/324) on instrumental conditions and analyte concentrations.

samples were found to be 30±6 pg/g and 347±52 pg/g (mean±S.D.) respectively (Table 1).

The sub-nanogram level of 5MT found in rat brain was much lower than those cited in previous reports [21,22], but is in agreement with the report of Bosin et al. [29], as well as that of Narasimhachari et al. [30], who could not detect, at nanogram levels, 5MT in the rat whole brain or hypothalamus using a

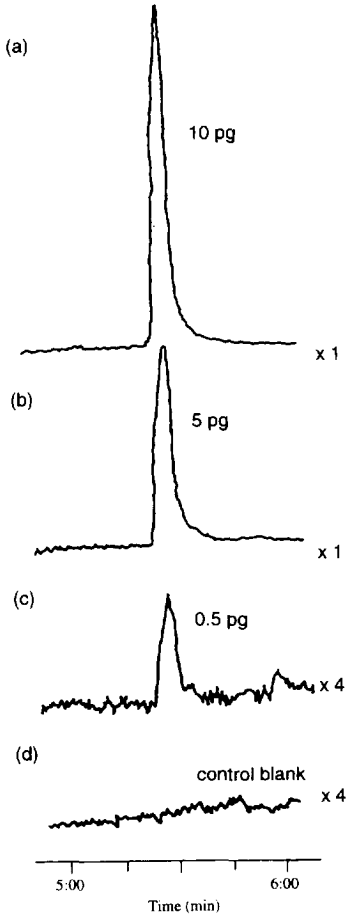


Fig. 3. GC–EC-NICI-MS–SIM chromatogram profile of 3,3′-spirocyclic pentafluoropropionyl indolenine derivative (*m/z* 321 ion) of N-[$^2H_3$]acetyl-5-methoxytryptamine (d_3-Ac-5MT): (a) 10 pg, (b) 5 pg, (c) 0.5 pg equivalent of 5MT and (d) control blank.

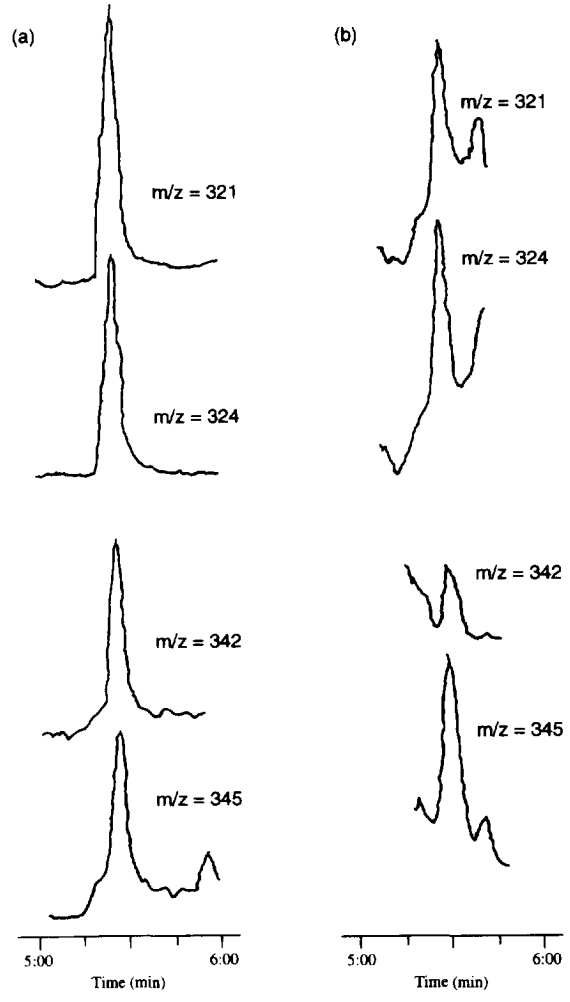


Fig. 4. Typical GC–EC-NICI-MS–SIM chromatogram profiles of 3,3′-spirocyclic pentafluoropropionyl indolenine derivative of N-[$^2H_3$]acetyl-5-methoxytryptamine (d_3-Ac-5MT) and N-[$^2H_3$]acetyl-5-methoxy-[$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine ($d_3$-Ac-$d_4$-5MT, internal standard) of (a) quail brain and (b) rat brain at *m/z* 321/324 and *m/z* 342/345.

GC–electron-impact or field ionization MS method. After conversion to the *o*-phthaldehyde derivative and analysis by thin layer chromatography/fluorescence spectrophotometry, Prozialeck et al. reported 120±90 ng/g of 5MT in the rat hypothalamus [22], but the abnormally high value could be attributed to other sources of fluorescence emissions derived from indolic compounds and proteins present in the biological matrix. Using GC–EI-MS, Green et al. reported low nanogram levels of 5MT (6.3±0.2 ng/g) in the rat hypothalamus, but the analysis was subjected to a possible artifact arising from the transacylation reaction of pentafluoropropionic anhydride with melatonin, especially when the sample

Table 1
Measurement of 5-methoxytryptamine (5MT) in whole brain samples

Sample	n[a]	Brain weight (mean± S.D.) (g)	5MT levels (mean±S.D.) (pg/g)
Rat	6	1.74±0.06	30±6
Quail	6	0.72±0.06	347±52

[a]Number of pooled samples, each consists of 4 rat brains.

contained a substantial amount of melatonin, giving a false high value [29,30]. A high level of melatonin in the rat hypothalamus has been demonstrated [42]. This possible artifact was avoided in the present study by using d_6-acetic anhydride in the N-acetylation of 5MT, thereby producing a deuterated 3,3'-spirocyclic indolenine derivative which yielded fragment ions with different m/z values compared to that of melatonin in the EC-NICI mass spectrum.

To our knowledge, this is the first report on the endogenous level of 5MT in the brain of an avian species, the quail. The 5MT level in quail brain is about 10-times higher than that found in the rat brain, a trend that was also observed for other methoxyindoles e.g. melatonin and N-acetylserotonin [40,42]. The variation observed in the 5MT levels may reflect differing rates of synthesis and/or degradation of 5MT in different species, though this remains to be investigated. Alternatively, different stages of biological development may contribute partly to the difference demonstrated.

By employing a more sensitive and specific analytical technique, namely EC-NICI-MS which had not been adopted in previous studies, we are able to demonstrate and measure the femtomole levels of 5MT in the rat and quail whole brain in the absence of a monoamine oxidase inhibitor. This ultra-sensitive GC–MS method could be applied to physiological studies involving the measurement of 5MT in the brain, such as the biosynthesis, regional and subcellular localization, and functional roles of 5MT in the mammalian central nervous system.

5MT has been used as a specific agonist for 5-HT1D receptors [14]. Thus, endogenous 5MT may act on receptors presently classified as 5-HT1D in tissues. It would be interesting to see if the 5-HT1D receptors are in fact 5MT receptors mediating the endocrine, paracrine or neurotransmitter function of 5MT secreted by the pineal gland and/or neural tissues [16].

Under an in vitro condition, 5MT is a precursor of a very potent hallucinating agent, δ-methoxytetrahydroharman [4] and a psychotomimetic agent, 5-methoxy-N,N-dimethyltryptamine [3]. In light of the potential of 5MT as the precursor of β-carbolines which are endogenous hallucinating agents in psychiatric disorders, further investigations of 5MT in the brain tissues may be rewarding.

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