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Determination of tryptamine in rat brain by gas chromatography–mass spectrometry*

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Abstract: Tryptamine (TA) occurs in trace levels in the brain, but its role in the central nervous system is not clear. However, there is evidence that TA may be a neuromodulator since it binds to specific binding sites in the brain. TA was measured as a diheptafluorobutyryl derivative in rat whole brain by capillary gas chromatography–mass spectrometry using negative chemical ionization (NCI) and single ion monitoring (SIM). d_4-TA was used as the internal standard. The ions m/z 532 and m/z 536 were monitored to identify TA and d_4-TA, respectively and to calculate the concentration of TA in rat whole brain which was found to be 0.19 ± 0.08 ng g^{-1} ($n = 8$). The results confirm the earlier TA concentrations measured by GC–MS using positive electron impact ionization. However, NCI improved the signal/noise ratio of the method increasing its sensitivity for TA.

Keywords: *Tryptamine; negative chemical ionization; gas chromatography–mass spectrometry.*

Introduction

Tryptamine (TA) occurs at trace levels in mammalian brain [1]. It has been suggested that this occurrence is fortuitous and not of physiological importance [2], and it is considered that TA is a by-product of the biosynthesis of 5-hydroxytryptamine (5-HT) [1]. The concentration of 5-HT is a thousand-fold higher than that of TA [3], but conversion by direct decarboxylation of L-tryptophan to TA has been demonstrated in brain tissue [4]. Regional heterogenity in the distribution of TA in the brain has been shown, with the highest levels occurring in the striatum [5].

The role of TA in the central nervous system is not clear. TA has been shown to have a role in the development of affective psychoses and schizophrenia [6], and it has also been suggested that it may act as a neuromodulator or a neurotransmitter in the brain [1]. This hypothesis is supported by recently published reports showing that TA binds to a specific binding site in the brain [7, 8].

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The concentration found for TA in the mammalian brain depends greatly on the analytical method used. Initial spectrofluorometric methods [9] were disturbed by tryptophan, the assays producing TA concentrations that were too high. The same is also true for the radioenzymatic assay [10]. TA has also been quantified as a dansyl derivative using thin layer chromatography [11] or mass spectrometry [12], the latter method yielding lower TA concentrations. Gas chromatographic mass spectrometric [13, 14] and HPLC [15, 16] methods have also been used for the quantification of TA in the brain. In this study we present a specific capillary column gas chromatographic mass spectrometric method for the quantification of TA in rat brain samples.

Experimental

Chemicals

D_4-Tryptamine was prepared by the method of Shaw *et al.* [17]. Tryptamine hydrochloride was purchased from Sigma. All other chemicals used were of analytical grade.

Preparation of sample

TA was measured in the whole brain of male Sprague–Dawley rats (180–200 g, Simonson Labs, CA, U.S.A.). The rats were decapitated and the brains were rapidly removed on ice, and weighed. Homogenization was performed in 5 volumes of ice cold 0.4 M $HClO_4$ containing 40 ng of internal standard d_4-tryptamine using a Polytronic homogenizer for 1 min.

After centrifugation at 15,000 g for 15 min, the pH of the supernatant was adjusted to a pH of 3.5 with 10 M NaOH, and the mixture was extracted on a Sep-Pak C-18 cartridge (Waters Associates, Milford, MA, U.S.A.). The cartridge was pre-wetted twice with 5 ml of acetonitrile and washed with two 5-ml aliquots of water. After adsorption of the sample, the cartridge was washed with two 1-ml aliquots of water and eluted by using three 0.5-ml aliquots of acetonitrile. The residue from the evaporation of the acetonitrile solution was dissolved in 0.5 ml of 0.1 M HCl, and the solution was then washed with 2 ml of diethyl ether, which was discarded. After adding 1 ml of a saturated potassium carbonate solution to the sample, it was purified with 2 ml of methylenechloride. The methylenechloride solution was evaporated and the residual water was removed by adding and evaporating 100 μl of methanol and 100 μl of benzene.

The residue was incubated for 2 h with 100 μl of 50% heptafluorobutyrylimidazole (HFBI, Regis Chemicals Co., Morton Grove, IL, U.S.A.) in benzene. The sample was dissolved in methylenechloride and washed four times with water. The evaporated sample was stored at −70°C and redissolved in *n*-heptane before analysis by GC–MS. Some samples were extracted without the Sep-Pak procedure, first using 1 volume of diethyl ether, and then after adjusting the pH to 12 with a saturated potassium carbonate solution, with 2 volumes of methylenechloride. The methylenechloride extract was then evaporated and derivatized as above.

When investigating the recovery of the procedure, the brains of three rats were pooled, homogenated as above, and 2.00 ng of TA was added to 6 samples of 2.4 ml of the homogenate, with three samples being used as controls. For determination of the standard curve, 40 ng of d_4-TA was added to 40 pg, 400 pg, 4 ng and 40 ng of TA in 0.4 M $HClO_4$ and the samples were extracted and derivatized as above.

Analysis by gas chromatography–mass spectrometry (GC–MS)

A modified Hewlett–Packard 5985 B gas chromatograph–mass spectrometer was used for recording the mass spectra and multiple ion chromatograms. The gas chromatographic separation was achieved with a bonded-phase fused silica capillary column (15 m, DB-5, J and W Scientific, Rancho Cordova, CA, U.S.A.) which was inserted directly into the ion source of the mass spectrometer. Helium was used as the carrier gas with a flowrate of 1 ml min^{-1}. The temperature of the dry injector (Allen Inc., Boulder, CO, U.S.A.) and the transfer line was adjusted to 250°C. After injection into the GC, the oven was kept at 100°C for 1 min, the temperature then increased to 250°C at the rate of 10°C min^{-1}.

Positive electron impact mode with an electron energy of 70 eV was used to collect the whole mass spectra for identification of TA and for the determination of its retention time. The multiple ion detection mass spectral data were acquired under the negative chemical ionization mode (NCI) with an electron energy of 230 eV. Methane was used as the reagent gas (1 Torr) and the temperature in the ion source was maintained at 100°C.

The intensities of the ions m/z 532 and m/z 536 were recorded for calculation of the TA concentrations in the samples by plotting the ion current ratios (m/z 532/536) against the concentration of TA in standard samples.

Results

Tryptamine yields two derivatives with HFBI, the diHFB derivative being the most intensive after incubation for 2 h. DiHFB-TA also showed better chromatographic properties than the monoHFB derivative. The retention times were 9.03 and 9.85 min for diHFB-TA and monoHFB-TA, respectively. With retention times of 11.80 and 11.47 min, tryptoline and methtryptoline did not interfere with the assay. The optimum incubation time for derivatization was 2 h, at which time a constant ratio between mono- and di-derivative quantities was reached. The NCI mass spectra of the diHFB-TA consists of two ions only, m/z 532 ($M-HF^-$, 100%) and m/z 512 ($M-2HF^-$, 31%).

The recovery of 2.0 ng tryptamine when added to blank pooled homogenate of rat brain (TA content 0.24 ± 0.09 ng g^{-1}) was found to be 2.20 ± 0.03 ng g^{-1}, ($n = 6$), which is 97.6%. The minimum detection level of assay for TA was 40 pg/g rat brain. TA was identified in the samples on the basis of retention time being identical with that of TA in the standard sample and that of d_4-TA analogue in the sample. The quantitation of TA was based on the ratio of ions m/z 532 and m/z 536 for TA and d_4-TA, respectively (Fig. 1). When Sep-Pak extraction was combined with purification of the sample by liquid extraction the concentration of 0.19 ± 0.08 ng g^{-1} (1.19 pmol g^{-1}, $n = 8$) was found for TA in rat whole brain.

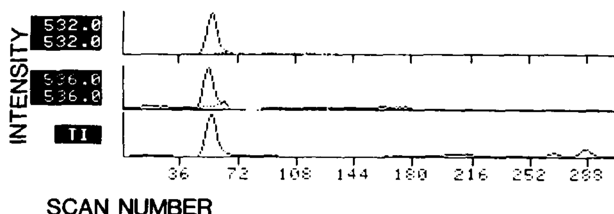

Figure 1
Selected ion chromatogram of diheptafluorobutyryltryptamine (m/z 532.0) and diheptafluorobutyryl-d_4-tryptamine (m/z 536.0) and the total ion current (TI) from a rat brain extract recorded by negative chemical ionization method with methane as a reagent gas.

Discussion

In neurochemical research, NCI mass spectrometry provides a combination of sensitivity and specificity which is superior to other analytical techniques [18]. Though many biologically active compounds show low electron affinities, appropriate derivatives with high electron affinity atoms or groups should make NCI mass spectrometry possible in most cases. In the present study TA was determined by the NCI technique as the corresponding diHFB derivative which improved the electron affinity of TA and made its analysis by GC possible. The results indicate that TA is present at trace levels in the whole rat brain. This is in agreement with earlier GC–MS results from the monitoring of positive ions produced by EI [13, 14].

The NCI technique leads to an improvement in the signal to noise ratio, since only compounds with electron capturing activity contribute to the signal current. Moreover, the NCI mass spectrum of TA is greatly simplified in comparison to the EI mass spectrum [13] of this compound, thus resulting in increased analytical sensitivity.

However, when using NCI the purification of the sample from interfering compounds becomes most important. When a sample of rat brain homogenate was extracted using the Sep-Pak cartridge and then derivatized with HFBI, an interfering compound was found in the NCI analysis. The compound coeluted with TA on GC-columns with different stationary phases and under various temperature programs. However, liquid extraction of the residue after Sep-Pak elution removed this interference. Liquid extraction has been used by other workers for the extraction of TA in homogenates of rat brain [13, 14]. However, when the Sep-Pak extraction was included as an additional step in the procedure the formation of an intense emulsion was avoided and the recovery of TA was improved.

Increased scientific interest in TA has been observed during the last few years after the discovery of specific binding sites of TA in the brain [8, 19, 20, 21], but the role of this compound in the central nervous system is not clear. TA turnover in brain is very rapid [22] and its local formation from L-tryptophan by decarboxylation [23] may suggest a neurotransmitter or a neuromodulator function in the brain. It is interesting that tryptolines, indolic compounds which might be derived from TA [24], show high affinity for the TA binding site [25]. Some tryptolines have been identified in rat brain at ten-fold higher concentrations [26]. The significance of TA and the tryptolines for CNS functions, as well as the interrelationships of these compounds, essentially remain an open field of study. The present method allows the measurement of TA at trace concentrations in brain samples.

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