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Brief Report

Identification of Dimethyltryptamine and *O*-Methylbufotenin in Human Cerebrospinal Fluid by Combined Gas Chromatography/Mass Spectrometry

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The hypothesis that hallucinogenic, methylated indolealkylamines such as dimethyltryptamine (DMT) and *O*-methylbufotenin (OMB) may be produced endogenously in psychotic patients has been pursued for 15 years (e.g., Mandell and Segal, 1973; Benington *et al.*, 1965). In this connection we have been concerned with the measurement of these compounds in human cerebrospinal fluid (CSF). These studies require a sensitive, reliable assay and in the past we have used a gas chromatographic (GC) method (Benington *et al.*, 1975; Christian *et al.*, 1975; Corbett *et al.*, 1978) which exhibited good sensitivity but suffered from a lack of specificity since the only criterion available for identification of the compounds was the retention time on the GC column. In this note we describe the initial application of a new highly sensitive and specific assay for DMT and OMB in human cerebrospinal fluid using combined gas chromatography/mass spectrometry (GC/MS) in a selected ion monitoring mode (see also Walker *et al.*, 1973; Oon and Rodnight, 1977; Oon *et al.*, 1977).

In the selected ion monitoring mode the mass spectrometer acts as a selective detector for the gas chromatograph wherein predetermined mass fragments, formed when a compound elutes from the GC column and enters the mass spectrometer, can be continuously monitored. The electron impact mass spectrum of both DMT and OMB (as heptafluorobutyryl derivatives, HFB) exhibits a fragment with mass to charge ratio (m/e) 58.2 as the base peak due to α-cleavage of the tertiary amino group. DMT and OMB can therefore be identi-

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fied as giving rise to peaks (corresponding to m/e 58.2) at the proper retention time. Additional specificity is obtained by simultaneously monitoring a second fragment present in the mass spectrum of DMT or OMB and ascertaining that this second fragment gives rise to a peak at *exactly* the same retention time as the 58.2 peak and that the ratio of the two mass peaks is identical to that obtained from a pure standard sample. For DMT and OMB determinations fragments at m/e 129.1 and m/e 159.1, respectively, were selected as confirming ions.

As a selective detector, the mass spectrometer can distinguish between a parent compound and a stable isotope derivative since the difference in atomic mass of the isotope is reflected in a different mass to charge ratio of any fragment produced in the mass spectrometer which contains the isotope. We have therefore synthesized $\alpha,\alpha,\beta,\beta$-tetradeutero DMT and OMB for use as internal standards in the present assay. α-Cleavage of these derivatives in the mass spectrometer yields a fragment with m/e 60.2 (containing 2 deuterium atoms) and can thus be distinguished from endogenous material. The standard is added to the CSF prior to any sample preparation, and quantitation is achieved by measuring in the final extract the ratio of the 60.2 peak area (corresponding to a known amount of added deuterated standard) to the 58.2 peak area (corresponding to endogenous compound). The use of a stable isotope derivative as an internal standard has the added advantages of (i) precluding the necessity of quantitative extraction and derivatization procedures, (ii) acting as a carrier and minimizing on-column losses, and (iii) serving to exactly define retention time in the gas chromatograph.

METHOD

Cerebrospinal fluid samples (approximately 5 ml) were obtained by lumbar puncture at the L3-4 level either from psychiatric patients or from patients undergoing lumbar puncture for spinal anesthesia prior to genito-urinary surgery. It was not possible to standardize the time of collection or to impose other controls at this time, e.g., diet, drug-free condition, etc. All samples were stored until processing at −76 C in acid-washed polycarbonate tubes. Prior to extraction and derivatization, 500 ng each of tetradeuterated DMT and OMB were added to each sample. The details of extraction and derivatization have been reported elsewhere (see Benington *et al.*, 1975; Christian *et al.*, 1975). Briefly, CSF was deproteinized by the addition of perchloric acid and then extracted with methylene chloride following basification with 45% KOH. The methylene chloride extract was derivatized with heptafluorobutyrylimidazole and the products dissolved in 1 ml of methylene chloride for subsequent analysis using a Hewlett-Packard 5985A GC/mass spectrometer. Aliquots of 1.5 μliter were chromatographed over a 4-foot X 2-mm i.d. glass column of 2% SP-2250 on 100-

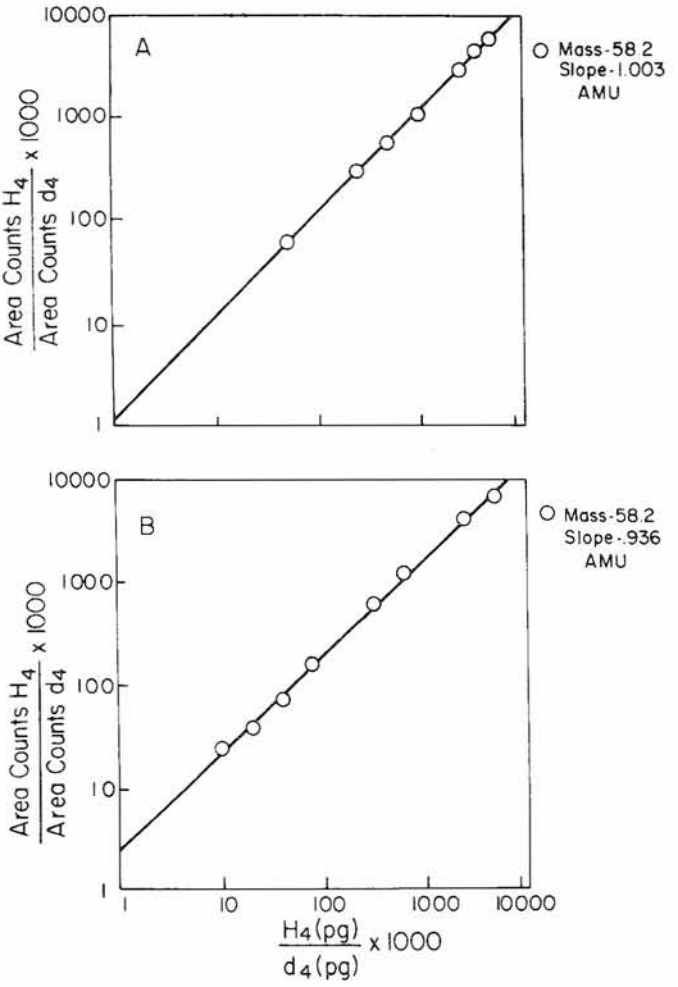

Fig. 1. (A) Calibration curve for DMT-HFB. The ratio of 58.2:60.2 peak areas (area counts) were determined for varying concentrations of unlabeled DMT-HFB from 10 pg to 1,000 pg/μliter in the presence of 200 pg/μliter α,α,β,β-tetradeutero DMT-HFB. The line was fitted to the data by a linear regression analysis. (B) Calibration curve for OMB-HFB. As in A, except that the concentrations of unrelabeled OMB-HFB ranged from 2 pg to 1000 pg/μliter.

120 mesh Chromosorb HP operated at a temperature of 150 C for 1 min followed by a programmed temperature increase of 10 C/min to 225 C. Helium was used as the carrier gas at a flow rate of 40 ml/min. Under these conditions DMT-HFB eluted with a retention time of 2.6 min and OMB-HFB with a retention time of 4.9 min.

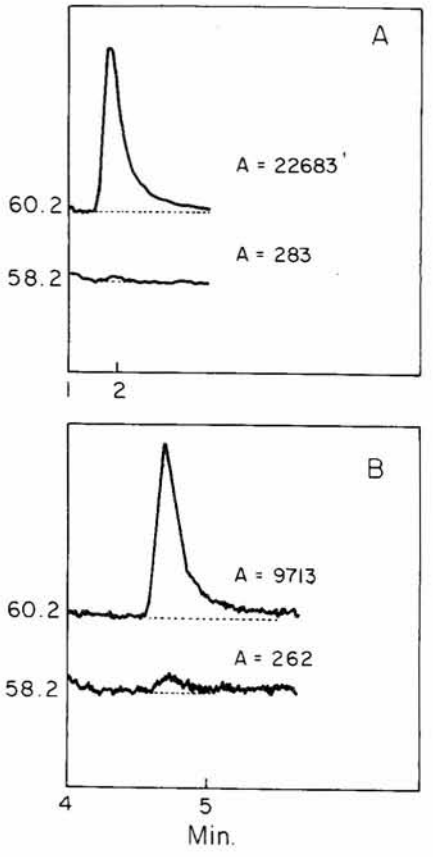

Fig. 2. (A) Sensitivity to DMT-HFB. The response of the mass spectrometer to 1 pg DMT-HFB in the presence of 220 pg tetradeuterated DMT-HFB injected on column. The mass spectrometer was run in the selected ion monitoring mode, simultaneously measuring m/e 58.2 (lower trace) and 60.2 (upper trace). The areas under the curves (A) were computed by the instrument data system as the sum of total ion current produced. (B) Sensitivity of OMB-HFB. Similar to A, showing the instrument response to 2 pg OMB-HFB.

For preliminary qualitative and quantitative determinations the effluent from the GC was monitored at m/e 58.2 and m/e 60.2 simultaneously. Thus a 58.2 peak with exactly the same retention time as the prominent 60.2 peak for internal standard d_4-DMT-HFB or d_4-OMB-HFB indicated the presence of endogenous material. Quantitative measurements were made by calculating the 58.2:60.2 peak area ratio obtained in the sample and comparing this value to a standard curve. The standard curves were obtained by measuring the 58.2:60.2 peak area ratio for varying concentrations of unlabeled DMT- or OMB-HFB in the presence of a fixed concentration of deuterated derivative. The data were fitted by a least squares analysis and plotted as the ratio of picograms proteo/picograms deutero compound *vs.* peak area ratio. The standard curves are reproduced in Fig. 1 and indicate the excellent linearity over a wide range of concentrations obtained with this method. As shown in Fig. 2 the absolute sensitivity of the

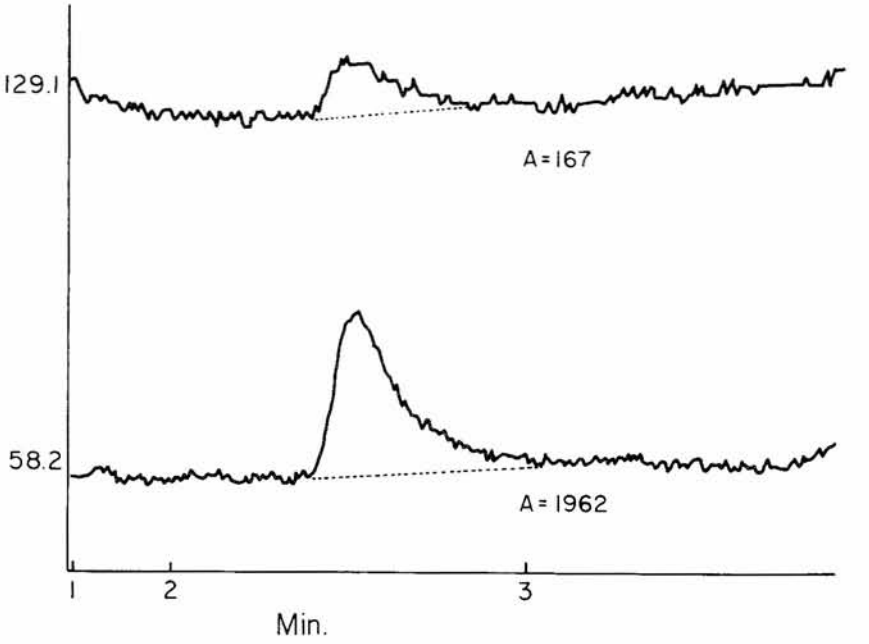

Fig. 3. Confirmation of DMT in cerebrospinal fluid, Patient 1. Simultaneous selected ion chromatogram of m/e 58.2 (lower trace) and m/e 129.1 (upper trace). Note the correspondence in retention times of the two peaks. The ratio of the peak areas was consistent with that determined from a standard sample of DMT-HFB. This sample was concentrated by a factor of 10 in order to measure the relatively low intensity m/e 129.1 signal.

technique is extremely high as indicated by the instrument response to 1 pg and 2 pg DMT-HFB and OMB-HFB, respectively, injected on column. The overall sensitivity of the method as presented here is approximately 70 pg DMT or OMB per ml of spinal fluid. Of course, this sensitivity could be increased within certain limits by using more spinal fluid or by concentrating the final extract. We have used the latter alternative in two cases (i.e., Patients 1 and 3) in order to absolutely confirm the presence of DMT by simultaneous selected ion monitoring of both m/e 58.2 and m/e 129.1. An example of the actual selected ion monitoring chromatogram for Patient 1 is shown in Fig. 3. As can be seen in this figure, the intensity of the m/e 129 peak is much reduced relative to the m/e 58 peak; hence the necessity for increased concentration. On the other hand, the level of DMT for one patient (2) was so high that the confirming 129.1 ion could be run without recourse to concentration of the sample. The chromatogram for this patient is presented in Fig. 4.

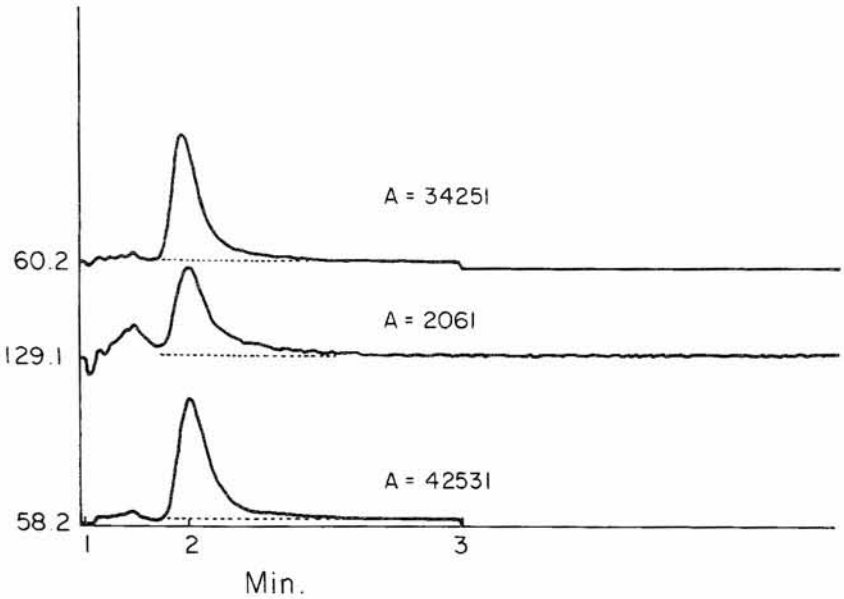

Fig. 4. Confirmation of DMT in cerebrospinal fluid, Patient 2. Similar to Fig. 3, except that the concentration of DMT in this sample was high enough to allow simultaneous monitoring of m/e 60.2, corresponding to added internal standard, plus m/e 58.2 and 129.1, the confirming ions for endogenous DMT.

Table I. Dimethyltryptamine Levels in Cerebrospinal Fluid

Patient	DMT ng/ml	Remarks
1	2.33[a]	Acute schizophrenic
2	100.37[a]	Bladder cancer, peripheral neuropathy, diffuse liver disease
3	3.63[a]	Chronic schizophrenic, 30 years' duration
4	7.21	Bladder surgery
5	0.35	Bladder surgery
6	0.85	Bladder surgery
7	<0.170	Lung cancer, cordotomy operation for pain
8	<0.158	Bladder surgery
9	<0.24	Bladder surgery
10	<0.19	Bladder surgery
11	<0.12	Acute catatonic schizophrenic

[a] Confirmed by monitoring m/e 129.1. See text for further details.

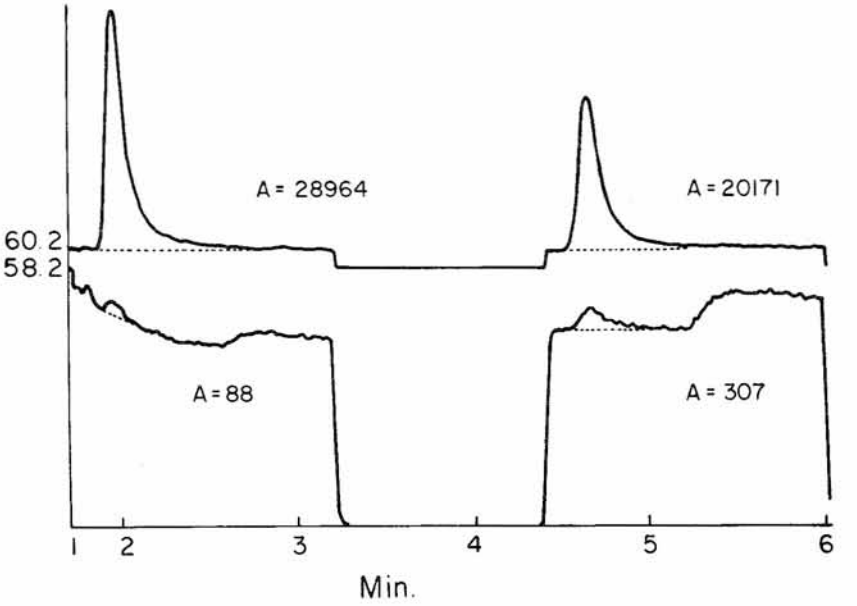

Fig. 5. OMB in cerebrospinal fluid, Patient 12. Simultaneous selected ion chromatogram of m/e 60.2 corresponding to added tetradeutero DMT-HFB or OMB-HFB and m/e 58.2, corresponding to endogenous material. The presence of OMB is indicated by the significant m/e 58.2 peak at the same retention time (4.7 min) as added standard. The presence of DMT is also suggested in this sample, but due to the very low intensity of the signal, we have not considered this to be positive.

RESULTS

The results of these studies on DMT levels in cerebrospinal fluid for 11 patients are summarized in Table I. Another patient (see Fig. 5) was found to be positive for OMB, but because of improper storage of this particular sample we were not able to run a confirming m/e 159.1 ion. Despite the preliminary nature of this study we can nevertheless make the following conclusions: (i) DMT is definitely present in a portion of cerebrospinal fluids from both surgical controls and psychiatric patients (lower limit of detection $\cong$ 70 pg/ml CSF); (ii) a remarkably wide range of concentrations was exhibited in those patients having detectable levels of DMT. The factors determining DMT levels in CSF are currently under investigation in our laboratory.

The gas chromatographic/mass spectrometric method described here is well-suited to the task of measuring these compounds in CSF, a task which places high demands on both sensitivity and selectivity. The high sensitivity of the technique is amply demonstrated by the instrument response to one pico-

gram of DMT-HFB injected on-column (see Fig. 2). Selectivity is gained relative to a strictly gas chromatographic procedure since the presence or absence of a fragment ion can be monitored in addition to the retention time. If only one ion is measured (e.g., 58.2 m/e) it is still possible that another compound other than DMT or OMB could elute at *exactly* the respective retention time and also have a 58.2 peak in the mass spectrum. By monitoring a second ion, however, the chances of misidentification become vanishingly small since four separate criteria must be fully met, namely, proper retention time, presence of mass 1, presence of mass 2, and the proper ratio between the two masses.

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