Life Sciences Vol. 12, Part II, pp. 475-478, 1973 Pergamon Press
Printed in Great Britain

GC-MS IDENTIFICATION OF BUFOTENIN IN URINE SAMPLES FROM PATIENTS WITH SCHIZOPHRENIA OR INFANTILE AUTISM

Nedathur Narasimhachari and Harold E. Himwich

Thudichum Psychiatric Research Laboratory
Galesburg State Research Hospital
Galesburg, Illinois 61401

(Received 1 March 1973; in final form 2 April 1973)

Summary

Bufotenin was found to be present in urine samples of schizophrenic patients and autistic children, but not of normal controls. The identification was based on TLC of the tertiary amine fraction using a specific *o*-phthalaldehyde spray, and also on GC. Unambiguous proof of the identity of bufotenin has now been provided by GC-MS analysis of the bufotenin-positive samples through its trimethylsilyl derivative.

The psychotomimetic properties of the three N:N-dimethyltryptamines, N:N-dimethyltryptamine (DMT), 5-methoxy-N:N-dimethyltryptamine (5-MeODMT) and bufotenin are well recognized. To demonstrate the psychotomimetic properties of bufotenin, it is necessary to eliminate the blood-brain barrier problem by intraventricular administration (1). For the past ten years the hypothesis of abnormal transmethylation involving catecholamines for O-methylation and indoleamines for N-methylation has been the subject of much research. The enzyme required for the N-methylation of tryptamine, N-methyltransferase, was first reported to be present in rabbit lung (2) and was subsequently found in human brain (1,3), lung (4) and serum (5).

In a series of studies the group from Galesburg State Research Hospital has demonstrated the presence of the three dimethylated tryptamines in blood samples from acute schizophrenics (6), and in urines from chronic schizophrenics under chemical stress (7,8), chronic schizophrenics with acute episodes, and drug-free schizophrenics on unrestricted diets (9). The identification of these compounds was based on TLC and GC methods using the tertiary amine fraction of the urinary extracts. More recently a sensitive and specific method for the

qualitative identification and the quantitation of bufotenin by TLC, using *o*-phthalaldehyde as a spray reagent was described (10).

We have reported the excretion of bufotenin in the urines of children with mental illness which had been diagnosed as infantile autism (11). In our continuing program of studies on schizophrenia we have identified bufotenin in urine samples from drug-free chronic schizophrenics on a rigorously controlled diet (unpublished data) by the TLC and GC methods reported earlier.

Methods

We have now identified bufotenin in urine samples from two of the autistic children and two drug-free chronic schizophrenics using the GC-MS method. The procedure was as follows: The tertiary amine fractions from urine samples of each of the four subjects which were positive for bufotenin (∿ 3 µg/24-hr sample) by both TLC and GC were heated with Regisil trimethylchlorosilane (TMCS) (20 µl) in sealed tubes at 100°C for 2 hr. Simultaneously a sample of authentic bufotenin was similarly heated (12). First the reference standard and then the urine samples were run on a GC-MS system, Varian MAT CH-7, Varian 2740 Gas Chromatograph, 2.5% OV-225 on GCQ 6-ft column, column temperature 190°C isothermal, He 30 ml/min, ionization potential 70 eV.

When the reference standard was run, the retention time of the single GC peak was noted (5 min 48 sec) and the mass spectrum was recorded. The spectra for the samples at the same retention time as the standard and the spectra of the peaks immediately preceding and following the bufotenin peak were also recorded. Two urine samples which were negative for bufotenin by TLC and GC were similarly treated.

Results and Discussion

Standard bufotenin gave a fully derivatized di-TMS-derivative and the mass spectrum showed the chacteristic molecular ion *m/e* 348, the base peak *m/e* 58, and fragments *m/e* 290, 202 and 73 (12). Three of the four samples (one from each of the two autistic children and one from a schizophrenic) showed a distinct peak both on total ion current (TIC) and flame ionization detector

(FID) with the same retention time as bufotenin TMS and the mass spectra of these were identical to that of the standard. The fourth sample (schizophrenic) showed a shoulder followed by a large peak. The mass spectrum of this shoulder again had all the characteristic peaks 348, 290, 202, 73, 58, while the specific peaks 348, 290, 202 and 58 were totally absent in the major peak. The GC peaks in the immediate region of bufotenin had no peaks at *m/e* 58, 290 and 348, thus ruling out the possibility of a background spectrum interfering with our identification. The spectra in all the samples showed that the GC peak was a single compound identical to bufotenin in retention time and mass spectrum. A typical mass spectrum from a sample is shown in Figure 1. Quantitation by the GC method was thus possible and the results of the spectrofluorometric method and GC analysis were comparable. The two samples which had previously been found negative for bufotenin by the TLC method were also negative by the GC-MS method.

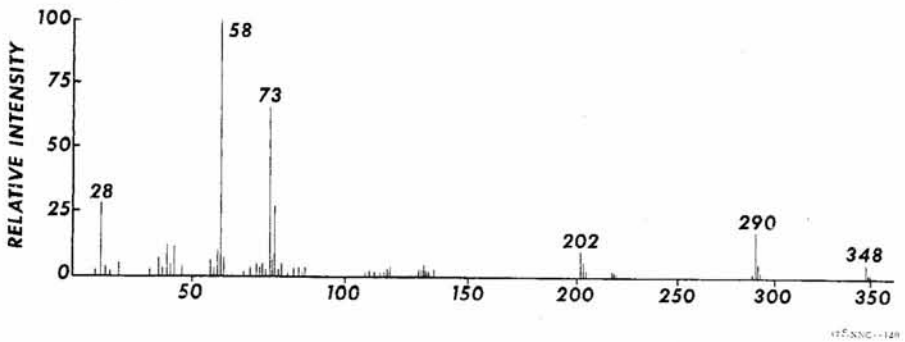

FIG. 1

Mass spectrum of bufotenin di-TMS from a urine sample.

We have established by the GC-MS method the unambiguous identity of bufotenin from urine samples in which we previously identified bufotenin by a specific TLC method using *o*-phthalaldehyde (10) and also by a GC method using TMS derivatives. We have also shown that the quantitative results of fluorometric and GC methods are comparable and, therefore, recommend the TLC method for the routine screening of urine samples. In the samples from four normal controls, the tertiary amine fraction did not show any bufotenin when the TMS derivatives were run on the GC-MS system (7). These samples were previously reported negative for bufotenin by TLC and GC methods.

Acknowledgement

We thank Judith Plaut and Katharine Leiner for their excellent technical assistance.

References

1. MANDELL, A. J. and MORGAN, M., Nature (New Biology) 230, 85-87 (1971).

2. AXELROD, J., Science 134, 343 (1961).

3. SAAVEDRA, J. M. and AXELROD, J., Science 175, 1365-1366 (1972).

4. MANDEL, L. R., AHN, H.-S., VANDENHEUVEL, W. J. A. and WALKER, R. W., Biochem. Pharmacol. 21, 1197-1200 (1972).

5. NARASIMHACHARI, N., PLAUT, J. M. and HIMWICH, H. E., Life Sci. 11 (Part II), 221-227 (1972).

6. NARASIMHACHARI, N., HELLER, B., SPAIDE, J., HASKOVEC, L., MELTZER, H., STRAHILEVITZ, M. and HIMWICH, H. E., Biol. Psychiat. 3, 21-23 (1971).

7. NARASIMHACHARI, N., HELLER, B., SPAIDE, J., HASKOVEC, L., FUJIMORI, M., TABUSHI, K. and HIMWICH, H. E., Life Sci. 9 (Part I) 1021-1032 (1970).

8. NARASIMHACHARI, N., HELLER, B., SPAIDE, J., HASKOVEC, L., FUJIMORI, M., TABUSHI, K. and HIMWICH, H. E., Biol. Psychiat. 3, 9-20 (1971).

9. NARASIMHACHARI, N., AVALOS, J., FUJIMORI, M. and HIMWICH, H. E., Biol. Psychiat. 5, 311-318 1972.

10. NARASIMHACHARI, N. and HIMWICH, H. E., J. Psychiat. Res. 9, 113-121 (1972).

11. HIMWICH, H. E., JENKINS, R. L., FUJIMORI, M., NARASIMHACHARI, N. and EBERSOLE, M., Journal of Autism and Childhood Schizophrenia 2, 114-126 (1972).

12. NARASIMHACHARI, N., SPAIDE, J. and HELLER, B., J. Chromatogr. Sci. 9, 502-505 (1971).