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Note

Mass fragmentographic quantification of urinary N,N-dimethyltryptamine and bufotenine

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The N,N-dimethylated metabolites of tryptamine and serotonin, dimethyltryptamine (DMT) and bufotenine, have been identified in human plasma and urine using a variety of analytical techniques including gas chromatography—mass spectrometry (GC—MS) [1—4]. An enzyme activity capable of forming the dimethylated compounds from the endogeneous non-methylated substrates has been demonstrated in several mammalian tissues [5—7].

The physiological significance of the N-methylating pathway of indolеamine metabolism and of the methylated end products remains unknown at present. Because of the known psychotropic properties of the dimethylated amines, their possible involvement in the chemical pathogenesis of mental disorders has received wide interest [8—11]. Comparative studies have been conducted on the activity of the N-methylating enzyme and on both the blood levels and the urinary excretion of the dimethylated amines in normal subjects and mentally disturbed patients. Somewhat contradictory findings have been reported. The dimethylated amines have been detected more frequently in the urine of psychotic patients than in the urine of normal controls [2, 12, 13]. Also, an elevated activity of the N-methylating enzyme has been claimed as present in the plasma of schizophrenic patients [14]. In other studies, however, no significant differences in either the urinary excretion or the blood levels of the dimethylated metabolites, or in the N-methylating enzyme activity have been detected [4, 8, 9, 15].

Several of these studies are based on the qualitative identification or a semiquantitative estimation of the dimethylated metabolites in the extracts of urine and plasma [1, 2, 9, 13]. A mass fragmentographic (MF) isotope dilution assay for DMT has been presented and applied to clinical studies [4, 15—17]. Also gas chromatography with nitrogen detection has been used for the quantifica-

tion of DMT [18]. Although mass spectrometry (MS) has been used for the identification of bufotenine in the urine [2], no quantitative MF technique has been so far presented.

In this paper we describe a quantitative isotope dilution assay for DMT and bufotenine based on MF. Both compounds can be assayed from the same sample as trimethylsilyl (TMS) derivatives. The deuterated analogues of the two compounds have been synthesized for use as carrier substances and internal standards. The isolation procedure for the dimethylated amines has been modified by introducing the non-ionic XAD adsorbent resin for extraction of the amines from aqueous solution. This facilitates the handling of large urine samples and applied together with a thin-layer chromatographic step reduces the contamination of the GC—MS instrument.

EXPERIMENTAL

DMT and bufotenine were obtained from Sigma (St. Louis, Mo., U.S.A.). The XAD resin (type 2, particle size 0.3—1.0 mm) was purchased from Serva, Heidelberg, G.F.R. Kieselgel G Type 60 was obtained from E. Merck, Darmstadt, G.F.R. The thin-layer chromatographic (TLC) plates (20 × 20 cm, 0.25 mm in thickness) were prepared with a Desaga TLC Spreader and prewashed with methanol—diethyl ether (4:1, v/v). The plates were activated at 110° for 60 min prior to use. The silylation reagents were the products of Pierce, Rockford, Ill., U.S.A. The deuterated analogues were prepared by an acid catalyzed exchange reaction [19]. Morning urine samples were obtained from 26 healthy medical students (10 females, 16 males). The samples, to which no preservatives were added, were frozen and kept at -18° until analyzed.

Isolation of the dimethylated amines

The internal standards (1 nmole of deuterated DMT and bufotenine) were added to 150 ml of the sample, which was then adjusted to pH 11 with 20% NaOH. If a precipitate was formed, it was removed by centrifugation. The adsorbent resin (5 g per 100 ml of sample) was added and the suspension was stirred mechanically for 20 min. The suspension was then poured into a column plugged with glass wool (1 cm I.D.). After the urine passed through, the retained resin was washed with 15 ml of water at pH 11. The amines were eluted from the resin with 20 ml of ethyl acetate at a flow-rate of 2 ml/min. The ethyl acetate was evaporated under nitrogen to 20—30 μl in a conical centrifuge tube and was then applied to silica gel G plates, which were developed in toluene—acetic acid—ethyl acetate—water (16:8:4:1, v/v). An area of 1 cm^2 was scraped around the site of application and the amines were eluted from the silica gel with two 1-ml portions of methanol, which was evaporated to dryness under nitrogen.

Derivatization and GC—MS

For GC—MS the amines were converted into TMS derivatives. The evaporation residue was reacted in a mixture of bis-trimethylsilyltrifluoroacetamide (BSTFA), pyridine, trimethylsilyldiethylamine (TMSDEA) and trimethylchlorosilane (TMCS) (100:100:30:1, v/v) at 85° for 30 min [20]. The volume

of the reagent was reduced under nitrogen to 20—25 μl and samples of 2—4 μl were injected into the GC—MS instrument.

A Varian Aerograph Model 1700 gas chromatograph coupled to a Varian MAT CH-7 mass spectrometer was used for GC—MS (electron impact ionization, 70 eV). The column was 1% OV-101 on 80—100 mesh Gas-Chrom Q operated at 190° for DMT and at 210° for bufotenine. Multiple ion detection (MID) was carried out with an Altema AL-5 detector. For standardization three samples of known composition as well as a water blank were carried through the entire analytical procedure with each set of analyses.

Creatinine was determined from the samples using the Jaffè reaction (normal values 0.9—1.7 g per 24 h).

RESULTS

Since relatively large volumes of urine had to be processed (at least 150 ml) to achieve reliable quantification of DMT, we have used the non-ionic XAD adsorbent resin to remove the dimethylated amines from aqueous solution. The amines could be eluted from the resin with a small volume of ethyl acetate (20 ml per 7.5 g of resin).

The bulk of the contaminating material moved readily with the solvent in TLC, whereas DMT and bufotenine possessed very low R_F values in the solvent selected and thus could be easily located at the site of application after the run.

The TMS derivatives of DMT and bufotenine were eluted as single, well defined GC peaks. The mass spectra of the two dimethylated amines as TMS derivatives are presented in Fig. 1. In the case of DMT the TMS group is attached to the indole nitrogen. Its spectrum has a molecular ion at *m/e* 260 and an intense fragment at *m/e* 202 resulting from side-chain cleavage between the α and β carbons. In the case of bufotenine the TMS groups are attached to the indole nitrogen and to the phenolic hydroxyl group. In its spectrum the molecular ion is found at *m/e* 348 and the fragment produced by the side chain cleavage at *m/e* 290.

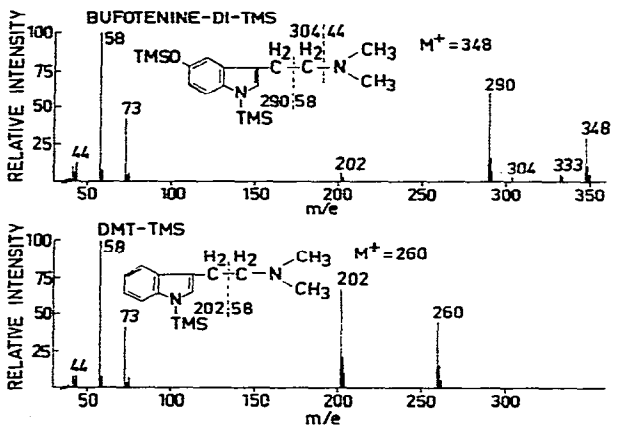

Fig. 1. Mass spectra and fragmentation pattern of DMT and bufotenine as TMS derivatives.

When the molecular ions of both compounds were used for MID, the runs were free from contaminating background peaks. The use of the fragments at *m/e* 202 and 290 resulted in occasional background interference. The detection limit of the assay varied slightly from day to day depending on the condition of the MS instrument. When the molecular ions were used for MID, DMT could be detected at a concentration of 0.1—0.15 ng/ml urine and bufotenine at 0.25—0.30 ng/ml urine. At least 150 ml of sample were required to achieve the detection limits reported above. The recovery after the entire analytical procedure was 40—45% for DMT and 25—30% for bufotenine. Eight analyses of a sample containing 0.5 ng/ml of DMT and bufotenine gave a coeffiecient of variation of 4.4% for DMT and 5.7% for bufotenine.

The results of quantitative analyses of urinary DMT and bufotenine of 26 healthy students are presented in Fig. 2. DMT was excreted at an average rate of 96 ng/g creatinine, the value being 105 ng for males and 81 ng for females. In case of bufotenine the excretion rate averaged 950 ng/g creatinine, 990 ng for males and 875 ng for females. If one exceptionally high value of DMT excretion is excluded, a mean excretion rate of 88 ng/g creatinine is obtained

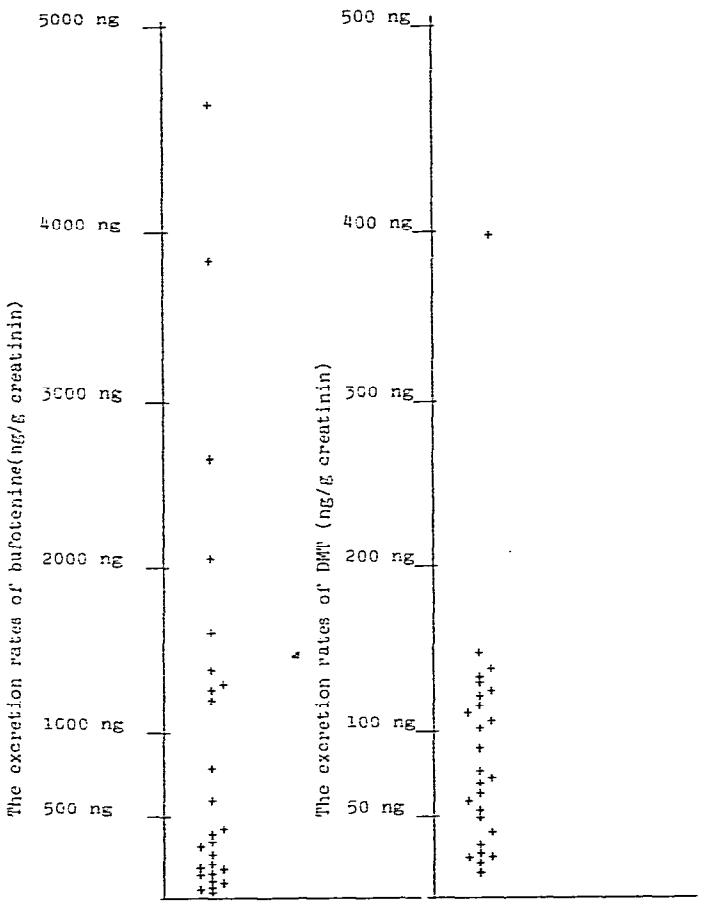

Fig. 2. The excretion rates of DMT and bufotenine calculated per g of urinary creatinine.

for males. After this exclusion the difference in the excretion rates of either DMT or bufotenine between the sexes becomes insignificant.

DISCUSSION

The most commonly employed isolation procedures for DMT and bufotenine include an initial solvent extraction step. Further purification is achieved either by back extraction into acidic aqueous solution and re-extraction into the organic solvent or by means of TLC. The initial solvent extraction step either requires large volumes of the organic solvent or reduction of the sample volume by vacuum evaporation or by lyophilization. The latter procedures, besides being laborious, lead to low yields due to decomposition and adsorption of the amines on to the laboratory ware. These problems could be overcome by employing the XAD adsorbent resin for the initial extraction of the amines from aqueous solution.

The urinary excretion of bufotenine determined with the present method far exceeds that of DMT. In contrast to the DMT values, which form a fairly homogeneous group, the bufotenine values have a very wide inter-individual variation with a majority at the lower end of the scale. Of the 26 subjects studied, 16 excreted less than 600 ng/g creatinine with a smaller cluster between 1000 and 2000 ng. A few higher values are observed between 2000 and 5000 ng. Whether the greater excretion of bufotenine as compared to DMT is due to differences in the synthesis of these compounds or differences in their elimination from human body is not known. However, experiments with parenterally administered DMT and bufotenine have been carried out [8, 21]. Only 0.07% of a dose of DMT was recovered in the urine, in the case of bufotenine the comparable figure was found to vary between 1 and 6% implying that a greater proportion of endogeneously synthesized bufotenine may be excreted unchanged in urine compared to DMT.

The DMT excretion rates reported earlier using GC—MS and GC with nitrogen detection [16, 18] are in reasonable agreement with those obtained with the present method. In the case of bufotenine, although estimates based on TLC techniques have been presented [1, 9, 13], a reliable quantitative method has been lacking so far. In the studies published on the comparison of bufotenine excretion in normal subjects and mentally disturbed patients, about half of the subjects investigated fell below the detection limits of the assays employed [1, 9, 13] and the possible differences between the low excretors of the two groups were consequently not detectable.

Even though the same enzymatic activity has been suggested as being responsible for the production of both DMT and bufotenine [5, 6], no correlation was observed between the excretion rates of these compounds. The availability of the non-methylated substrates, in addition to differences in the degradative metabolism, may explain this disparity.

Because of the low urinary recovery of the parenterally administered DMT and bufotenine, doubts have been expressed as to whether determination of the free amines as such can give any valid information about the metabolic significance of these compounds. However, since the metabolites of the degradative pathway (3-indole acetate, 5-hydroxyindole acetate) are not specific for the

N-methylated indoleamines, the determinations of these amines, in addition to metabolic studies using labelled tracers, appear to be the only way of obtaining information about their quantitative metabolism in the human body. Whether determinations of blood levels rather than the urinary excretion of DMT and bufotenine would be more useful, is doubtful, since the mode of release of these compounds into the circulation is not yet known. If they are released into the circulation sporadically, as may well be the case, the blood levels would vary greatly as a consequence and their determinations from single blood samples would be of limited significance. Frequent or continuous sampling is prevented by the large volumes of plasma needed for quantitative determinations. The urinary excretion, even though it represents only a fraction of the total amount of the compound metabolized, gives a summation over a longer period and can serve as a more reliable indicator of the overall quantitative metabolism of DMT and bufotenine.

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