

ORIGINAL ARTICLE

Potentially hallucinogenic 5-hydroxytryptamine receptor ligands bufotenine and dimethyltryptamine in blood and tissues

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Abstract

Bufotenine and N,N-dimethyltryptamine (DMT) are hallucinogenic dimethylated indolethylamines (DMIAs) formed from serotonin and tryptamine by the enzyme indolethylamine N-methyltransferase (INMT) ubiquitously present in non-neural tissues. In mammals, endogenous bufotenine and DMT have been identified only in human urine. The DMIAs bind effectively to 5HT receptors and their administration causes a variety of autonomic effects, which may reflect their actual physiological function. Endogenous levels of bufotenine and DMT in blood and a number of animal and human tissues were determined using highly sensitive and specific quantitative mass spectrometric techniques. A new finding was the detection of large amounts of bufotenine in stools, which may be an indication of its role in intestinal function. It is suggested that fecal and urinary bufotenine originate from epithelial cells of the intestine and the kidney, respectively, although the possibility of their synthesis by intestinal bacteria cannot be excluded. Only small amounts of the DMIAs were found in somatic or neural tissues and none in blood. This can be explained by rapid catabolism of the DMIAs by mitochondrial monoamino-oxidase or by the fact that the dimethylated products of serotonin and tryptamine are not formed in significant amounts in most mammalian tissues despite the widespread presence of INMT in tissues.

Key Words: *Blood, bufotenine, dimethyltryptamine (DMT), human, mass spectrometry, rat, tissues*

Introduction

The hallucinogenic N,N-dimethylated metabolites of serotonin and tryptamine (DMIAs), bufotenine (N,N-dimethyl-5-hydroxytryptamine) and DMT (N,N-dimethyltryptamine)

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have been identified in human urine using gas chromatography-mass spectrometry (GC-MS) [1–4] and high-performance liquid chromatography-mass spectrometry (HPLC-MS) [5]. Bufotenine is a normal constituent of human urine and its excretion is elevated in psychiatric patients [4], especially in those with paranoid symptoms [6,7]. DMIs are catabolized by monoamine oxidase (MAO). Inhibition of MAO by an irreversible inhibitor, nialamide, increases urinary bufotenine excretion in man [8].

The enzyme indolethylamine N-methyltransferase (INMT) [9], which is capable of forming DMIs and is present in several mammalian tissues, has recently been cloned from rabbit [10] and human [11] lung. It is present in the majority of stromal and epithelial cells of organs related to the autonomous nervous system, but absent from neurons and striated muscle cells (Kärkkäinen et al., unpublished observations, 2004).

The function of INMT and the physiological significance of the N-methylating pathway of indolethylamine metabolism remain unknown at present. Because of the known psychotropic properties of the DMIs, their possible role in the chemical pathogenesis of mental disorders has received wide interest. However, since INMT is predominantly present in peripheral tissues, its main physiological function is presumably non-neural. Bufotenine and DMT are potent 5-HT receptor ligands and their administration causes a variety of autonomic effects, which may reflect their actual physiological function. The hallucinogenic actions of the DMIs are mediated most likely via an agonist or partial agonist action at the 5-HT₂ receptor [12].

The same tissues that contain INMT often contain enzymes that catabolize DMIs, including MAO. Therefore, only a fraction of intracellularly formed bufotenine or DMT are expected to be transported to blood, from where they are rapidly eliminated [13,14].

The presence of DMIs in human or animal tissues and body fluids other than urine has not been conclusively established. In attempts to find DMIs in blood the methodology, including the mass spectrometric methods [15,16], has lacked the necessary sensitivity and/or specificity.

In the present study we have applied high-performance liquid chromatography-tandem mass spectrometry (HPLC/ESI-MS/MS) using multiple reaction monitoring (HPLC/MS/MS) to measure bufotenine and DMT in human blood and in a variety of human and animal tissues. Using MS/MS and two daughter fragments for monitoring, superior specificity is achieved in addition to increased sensitivity obtained through purification of the samples by preparative HPLC.

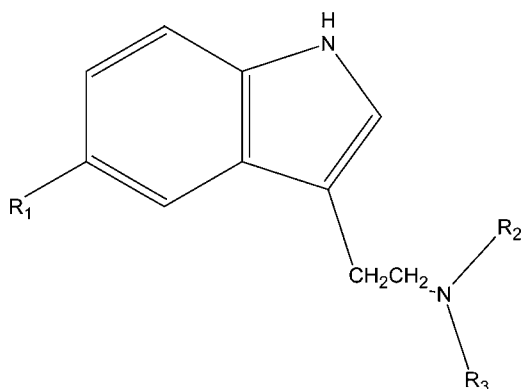
Material and methods

Materials

Bufotenine (N,N-dimethyl-5-hydroxytryptamine) was purchased from Sigma Chemical Co. (St. Louis, Mo., USA) and Cerilliant Corporation (Round Rock, Tex., USA), N,N-dimethyltryptamine (DMT) from Sigma and 5-methoxy- N,N-dimethyltryptamine (5-MeODMT) from Acros Organics (Fisher Scientific Ltd., UK).

The chemical structures of the analytes are presented in Figure 1.

Solvents used for HPLC were from Rathburn (Scotland, HPLC grade). HPLC grade water (MilliQ water purification system, Millipore Corp., Bedford, Mass., USA) was used for preparation of all aqueous standard solutions, dilutions and mobile phases. Oasis HLB extraction cartridges were from Waters (Milford, Wash., USA).



Serotonin $R_1=OH$, $R_2=R_3=H$

N,N-dimethylserotonin (Bufotenine) $R_1=OH$, $R_2=R_3=CH_3$

Tryptamine $R_1=R_2=R_3=H$

N,N-dimethyltryptamine (DMT) $R_1=H$, $R_2=R_3=CH_3$

5-methoxy-N,N-dimethyltryptamine (5-MeODMT) $R_1=CH_3O$, $R_2=R_3=CH_3$

Figure 1. Structures of the indolethylamines.

Standard solutions

Standard stock solutions were prepared in methanol (Rathburn). The solutions were diluted to appropriate concentrations with 0.2% HCOOH in methanol or methanol/water (1:1) for blood and urine analyses or with PBS (phosphate buffered saline) solution for testing of the solid phase extraction. The solutions were stored at $-18^{\circ}C$ in the dark.

Patients

Serum and EDTA (ethylene diamine tetra-acetic acid) plasma samples were obtained using routine clinical chemical methods from 137 unselected patients from the surgical, medical and psychiatric wards of Peijas Hospital, HUCH (Vantaa, Finland), and from the Departments of Obstetrics and Gynecology, HUCH (Helsinki, Finland). Samples of lung and kidney tissue, taken during surgery, were obtained from the surgical clinic of Peijas Hospital. The study was approved by the Committee on Research Ethics at Peijas Hospital.

Storage of human blood, urine and tissue samples

Serum and plasma samples were stored in plastic tubes and kept for a maximum of 48 h at $4^{\circ}C$ in the dark, after which they were frozen and kept at $-20^{\circ}C$ until analyzed. No decomposition of bufotenine DMT or 5MeODMT, added to plasma or serum samples, was observed after 30 min at $20^{\circ}C$ followed by storage for 24 h at $4^{\circ}C$. Samples of macroscopically normal lung and kidney tissue, taken during surgery from removed organs,

were cut into small pieces and frozen at -20°C within 20 min. Urine samples (5–10 ml) were stored in 10-ml plastic tubes at -20°C without preservatives.

Rat and rabbit tissues

Male Sprague-Dawley rats (4 months old, weighing 440–540 g, Harlan, The Netherlands) were given the irreversible MAO A/B inhibitor pargyline HCl (50 mg/kg, i.p., Research Biochemicals International) once a day for two days preceding sampling. Rats were sacrificed with CO_2 after which the brain was removed from the skull and liver, kidneys and lungs were dissected. All tissue samples were frozen on dry ice immediately after the dissection and stored at -70°C until analysis. The bodies of the rats were stored at -20°C . Freshly dissected tissue samples of rabbits were immediately frozen and stored at -20°C .

Preparation of tritiated standards

Bufotenine and DMT were tritiated by refluxing in tritiated acetic acid solution for 20 h, followed by evaporation of the solvent. For back-exchange of the labile protons the residue was dissolved in ethanol. The products were further purified by chromatography on silica followed by chromatography using Oasis HLB columns.

The tritiated standard stock solutions were diluted into a concentration of 1:10000 of the original solution with methanol. Samples spiked with 100 μl of this tritiated standard solution were employed to optimize the procedures of extraction and HPLC purification of plasma, serum and tissue samples.

Preparation and extraction of serum, plasma, urine and tissue samples

On the basis of testing solid phase (C18, HCLX, Oasis MCX, Oasis HLB) and liquid-liquid extraction on different matrices (PBS, serum, plasma, urine, human lung and liver) Oasis HLB column was selected.

Plasma and serum. To 1 ml EDTA plasma or serum sample, 25 μl working internal standard solution (5-methoxy-N,N-dimethyltryptamine, 139 $\mu\text{g/l}$) and 1 ml 4% NH_4OH were added.

Urine. To a 5 ml urine sample 50 μl of the internal standard solution and 5 ml 4% NH_4OH were added. The sample was vortexed and centrifuged at 3500 rpm for 10 min and the supernatant collected.

Tissues. Thawed samples (0.5–1.5 g) were weighed and 25 μl of the internal standard solution (139 $\mu\text{g/l}$) plus 7 ml ice-cold 0.1 M HClO_4 (containing 0.02% ascorbic acid) were added. The sample was homogenized with an Ultra Turrax for 30 s. The homogenate was centrifuged for 30 min at 4°C and 10,000 rpm (Jouan Br4I centrifuge). The supernatant was transferred to a screw-capped plastic centrifuge tube and 7 ml 4% NH_4OH was added.

Extraction. The samples were loaded onto columns (Oasis HLB 1 cc, 30 mg cartridge for serum and plasma, and 3 cc, 60 mg cartridge for urine and tissues), which had been conditioned and equilibrated by adding 1 or 2 ml methanol and 1 or 2 ml water. Each cartridge was washed two times with 1 or 2 ml 5% methanol+2% NH_4OH followed by 1 or

2 ml 50% methanol+2%NH₄OH. The compounds were eluted twice with 0.75 or 1.5 ml methanol. The flow rates were 1 ml/min for 1 cc cartridges and 2 ml/min for 3 cc cartridges. Samples were evaporated to dryness (at approximately 40°C) under a gentle stream of nitrogen. The resulting residue was reconstituted with 60 µl methanol. A sample volume of 10 µl was injected into the LC-MRM instrument, for analysis. Alternatively, the sample was subjected to further purification by preparative HPLC.

Purification of tissue and blood samples by preparative HPLC

Preparative HPLC was performed using an LKB 2150 HPLC pump equipped with a 50 µl loop injector, and a Supelcosil LC-SCX, 5 µm (Supelco, 25 cm × 4.6 mm ID) column. The mobile phase consisted of 60% methanol and 40% 0.083 M ammonium acetate (pH 4.4) at a flow rate of 1 ml/min. Absorbance at 220 nm was monitored with an ABI Analytical Kratos Spectroflow 783 detector.

Fractions containing bufotenine (4.25 ml) and DMT plus internal standard (9.5 ml) were collected and evaporated to dryness (at approximately 40°C) under a gentle stream of nitrogen. The resulting residue was reconstituted with 100 µl methanol/water (1:1)+HCOOH (0.2%), transferred to a low-volume insert in the HPLC autosampler vial and 10 µl was injected into the LC-ESI-MS/MS instrument.

Analysis of heparinized plasma samples was not successful because of impurities interfering with LC-MRM analysis and EDTA plasma was therefore employed.

HPLC/ESI – MS/MS

The HPLC system used was an HP1100 series liquid chromatography system, consisting of a degasser (G1322A), binary pump (G1312A), automatic sampler (G1313A) and a column compartment (G1316A) (Hewlett Packard, Palo Alto, Calif., USA). The HPLC system was connected through an A-102X frit guard column (Upchurch Scientific Inc., Oak Harbor, Wash., USA) to an XTerra MS C₁₈ 3.5 µm 2.1 × 100 mm analytical column (Waters). Isocratic elution was carried out in order to shorten analysis time or with a programmed flow rate in order to achieve narrower peaks. The isocratic solvent was water:methanol (70:30) containing 0.1% acetic acid. The flow-rate program was 0 min/(60 µl/min)–3 min/(120 µl/min)–5 min/(120 µl/min)–8 min/(60 µl/min)–10 min/(60 µl/min). Flow programming of the HPLC allowed an analysis to be made in only 10 min. The chromatographic peaks obtained were narrow. For example, the half height width of a 250 fg/µl standard of bufoteine was 15 s. The fast analysis time allowed the use of solvent blanks between every sample and standard to monitor possible crossover contamination. The column temperature was kept at 25°C.

The analytical column was connected without splitting to a Micromass Quattro II tandem quadrupole mass spectrometer (Waters, Micromass Atlas Park, Manchester, UK), which is operated with MassLynx version 3.5 software. An electrospray inlet was used to ionize the analytes.

The tuning parameters for the electrospray inlet and the mass spectrometer were as follows: capillary sprayer voltage 3.3 kV, HV lens 0.5 kV, sampling cone voltage 24 V, skimmer offset 4V, skimmer 0V and RF lens 0.1V. Nebulizing temperature was 270°C and the Z-spray source temperature was 90°C. Drying gas and nebulizer gas, both of which were nitrogen generated from a Whatman Model 75–72 nitrogen generator, were set to 250 l/h and 15 l/h, respectively.

The experiments were done in the positive ion mode using multiple reaction monitoring. The MRM experiments allow selected MS/MS fragments to be monitored and are thus more selective than selected ion recording (SIR), a major advantage when trying to determine analytes from biological samples with quadrupole instruments. The precursor and product ions of interest, bufotenine, DMT and 5MeO-DMT, used in MRM experiments were (exact mass):

Compound	Precursor ion	Product ion 1	Product ion 2
Bufotenine	205.134	160,076	58,066
DMT	188.131	144,081	58,066
5-MeO-DMT (std)	219.150	174,092	58,066

In the MRM experiments only integers have been used. The acquisition dwell time for every transition precursor to product ion was set to 300 ms. Grade 4.8 argon was used as collision gas. The gas pressure at the collision path was kept at 1.3×10^{-3} bar. The collision energy was 18 eV for all transitions.

Detection limit and quantitative analysis

The detection limits (LODs) for bufotenine and DMT for different matrices (serum, plasma tissue) were calculated from spiked samples. To qualify as a positive result, the peak height of both product ions had to exceed three times the noise level in the same LC/MRM analysis. Quantification was based on the average peak areas of the two product ions and those of the internal standard.

Results

Bufotenine and DMT in human serum, plasma, stool and urine

Using the simple one-step purification procedure for serum or plasma samples ($n=66$) the detection limit ($S/N > 3$, sample size 1 ml) was 300 ng/l for bufotenine and 200 ng/l for DMT. By using preparative HPLC after the initial extraction, the disturbing impurities could be largely removed. The detection limit (sample size 1 ml) could thus be lowered to 50 ng/l for bufotenine and 30 ng/l for DMT ($n=35$), and, when the sensitivity of the LC/ESI-MS/MS instrument was optimized, to 10–20 ng/l for bufotenine and 5–10 ng/l for DMT ($n=36$). The detection limits for both bufotenine and DMT were 50 ng/kg and 100 ng/l for stool and urine, respectively.

Bufotenine and DMT were not detected in any of the 137 serum or plasma samples from hospital patients (Table I) using LC/ESI-MS/MS.

The stool of healthy control persons (Table I) contained very large amounts of bufotenine ($n=13$, median 17 $\mu\text{g/kg}$, range 1.0–180 $\mu\text{g/kg}$). The levels were more than 10 times the concentration of bufotenine in spot urine samples of control persons ($n=9$, median 1.4 $\mu\text{g/l}$, range <0.05 –9.1 $\mu\text{g/l}$).

DMT was detected in one stool specimen (0.13 $\mu\text{g/kg}$) and in none of the urine samples.

Bufotenine and DMT in kidney, lung, liver and brain

The results of bufotenine and DMT analyses of kidney, lung, liver and brain of rats (pretreated with an MAO inhibitor), of normal human kidney and lung as well as of rabbit liver are presented in Table II. Only analyses performed at the highest sensitivity (detection

Table I. LC-ESI-MS/MS analysis of bufotenine and dimethyltryptamine (DMT) in human blood, urine and stool using a one-step purification procedure* and prepurification with HPLC**. Limit of detection***.

Serum*		Plasma**		Plasma**		Urine**		Stool**	
Patients, <i>n</i> =66, ng/l		Patients, <i>n</i> =35, ng/l		Patients, <i>n</i> =36, ng/l		Healthy personnel, <i>n</i> =9 µg/l, median (range)		Healthy personnel, <i>n</i> =13 µg/kg, median (range)	
Bufotenine <300***	DMT <200***	Bufotenine <50***	DMT <30***	Bufotenine <20***	DMT <10***	Bufotenine 1.4 (<0.05–9.1)	DMT <0.1	Bufotenine 17 (1.0–180)	DMT 0.13, <i>n</i> =1 <0.05, <i>n</i> =12

Table II. LC-MRM analysis of bufotenine and dimethyltryptamine (DMT) in tissues.

	Kidney		Lung		Liver		Brain	
	ng/kg tissue		ng/kg tissue		ng/kg tissue		ng/kg tissue	
	Bufotenine	DMT	Bufotenine	DMT	Bufotenine	DMT	Bufotenine	DMT
Rat tissues								
(MAO-inhibitor treatment)								
Rat 1	<10	12	34	22	39	6	29	10
Rat 2	<20	16	40	12	22	<10	17	15
Human tissues								
Patient 1	15	15						
Patient 2			<20	14				
Rabbit tissues								
Rabbit 1					<20	<10		

limit, $S/N > 3$, 10–25 ng/kg) are included. In rats treated with an MAO inhibitor, low but measurable concentrations of bufotenine and DMT were found in all tissues studied, and most consistently in brain. Low concentrations of bufotenine and DMT were also detected in human kidney. In human lung tissue, only DMT was detected. No concentrations of bufotenine or DMT were found in rabbit liver.

Discussion

In the present study, we have used highly sensitive assays to analyze bufotenine and DMT in body fluids, excretions and tissues using LC-MRM, the best method presently available for a quantitative analysis of low molecular weight metabolites. The LOD of the present method is more than 10 times lower than the LOD in our earlier work [5] and that of Barker et al. [15].

We initially used one-step purification with Oasis HLB extraction cartridges for serum and tissue samples before LC-MRM, but the sensitivity of the method was not satisfactory. Therefore we added a purification step by including preparative LC to remove impurities. The procedure is tedious and (in the case of bufotenine) less accurate since bufotenine and the internal standard are not recovered in the same fraction. Better accuracy can, as we have shown for urinary bufotenine [2], be obtained by employing a deuterated internal standard.

After adding the preparative LC step, the sensitivity was not limited by impurities from the sample but was affected by daily variations of the condition of the MS instrument and by carry-over effects from standards at both LC-MRM and preparative LC stages. The latter could be largely abolished by using as low amounts of standard solutions as possible for determining the RF values, and by using sufficient number of washes after the standards. Finally, we discontinued the use of standards that could cause carry-over effects in preparative HPLC in the columns that were used in actual analyses.

One of the main goals of the present work was to develop an LC/ESI-MS/MS method suitable for measuring blood levels of DMIs to corroborate earlier findings [17] of the presence of DMT in plasma and to facilitate patient studies using plasma instead of urine. We were, however, unable to detect bufotenine or DMT in any of the blood samples analyzed even at the highest level of sensitivity (< 20 ng/l for bufotenine and < 10 ng/l for DMT).

Urinary bufotenine is thought to originate from somatic tissues containing INMT, from where it is transported via blood to the kidney and filtered into urine. However, the following calculations make this assumption unlikely.

If one assumes that the major proportion of bufotenine and DMT is not bound to plasma proteins, their average concentrations would be 200-fold higher in urine than in plasma, if no excretion in the tubuli takes place. A urinary bufotenine level of 5 μ g/l (close to the upper reference limit of 5 μ g/g creatinine [2,4]) would thus correspond to an average level of 20–30 ng/l in plasma.

In our recent work [5] urinary bufotenine levels higher than 5 μ g/l were found in about 10% of patients (psychiatric, surgical and medical) and in two patients urinary levels of 10–20 μ g/l (corresponding to expected average plasma levels of 50–100 ng/l) were found. Although our detection limit for bufotenine was, at maximal sensitivity, of the order of 20 ng/l, we found no bufotenine in any of our 36 serum and plasma samples analyzed. Neither did we detect bufotenine in the plasma samples analyzed with somewhat lower

sensitivities (detection limit 50 ng/l, $n=35$, detection limit 300 ng/l, $n=66$). Thus the absence of bufotenine in all the plasma and serum samples contravenes the notion that urinary bufotenine is derived from glomerular filtration.

If bufotenine and DMT were, to a significant extent, bound to proteins, the expected plasma levels would be even higher, since the glomerular filtration rate is determined by the free metabolite fraction. In our method, both free and protein-bound fractions are measured together.

We also expected to find high DMIA content in tissues that are rich in INMT, but this was not the case. We found small amounts of bufotenine and DMT in tissues of rats pretreated with an MAO inhibitor, in human kidney and, in the case of DMT, in human lung. In rat tissues the highest DMIA concentrations were found in lung, which is known (in man and rabbit) to contain high levels of INMT, but also in brain tissue. The presence of both DMIA in the brain was somewhat surprising, since the brain is known to lack INMT [11]. The result can be explained by accumulation in the brain of lipophilic bufotenine and DMT formed in peripheral tissues and by the slow catabolism of DMIA in the brain. This finding should, however, be corroborated in future studies.

The small amounts of bufotenine found in kidney tissue can be thought to originate from urine present in the tubuli, since high levels of bufotenine are found in human urine. The presence of DMT, however, cannot be similarly explained, because only traces of DMT are found in urine.

Tissue samples from animals as well as human tissue samples taken during the operation were frozen with minimal delay. The present negative results of DMIA in tissues and plasma do not completely preclude the possibility of DMIA synthesis in these tissues since the same tissues that contain INMT also often contain MAO, by which endogenous bufotenine and DMT could be effectively degraded. MAO is a mitochondrial enzyme and does not occur in urine and stools. In this work we used an MAO inhibitor to prevent the oxidative degradation using rat as a test animal, but again only trace amounts of the DMIA were found in the tissues. This animal was selected because of the possible usefulness of rats in physiological studies of DMIA function. Neither INMT nor DMIA have, thus far, been identified in the rat, and the negative results should, because of the species difference, be interpreted with caution.

The reason for the low levels or absence of bufotenine and DMT in tissues might also be the fact that they are not formed at all owing to, for example, the lack of the necessary substrates, serotonin and tryptamine. The presence of bufotenine in urine contravenes the latter alternative, at least as far as the kidney is concerned. Kidney tissue contains high concentrations of INMT and could thus be the source of urinary bufotenine.

The presence of large amounts of bufotenine in stools is a completely new finding, and suggests that this 5HT ligand could play a part in intestinal function. The amounts found were very high, on a weight basis of more than 10-fold those in urine samples analyzed for comparison and also in urine samples analyzed earlier [2,4].

We suggest that fecal bufotenine originates from intestinal epithelial cells. The intestinal bacterial synthesis or dietary origin of bufotenine and DMT cannot be excluded with certainty, although the presence of these amines in food or of N-dimethylating bacteria in stools has not been demonstrated. As shown by immunohistochemistry (Kärkkäinen, et al., unpublished observations, 2004) all glandular epithelial cells of the small and large intestine are rich in INMT. The same cells also contain large amounts of serotonin, and catabolism in feces, in contrast to intestinal epithelial cells containing MAO, is probably minimal. However, if feces were the source of urinary bufotenine, it would have to be

transported to the kidneys via blood, and should have been detectable in at least some of our plasma or serum samples.

Conclusions

To find clues to the biological function of the pharmacologically active indolethylamines, bufotenine and DMT, we have used a highly sensitive and specific mass spectrometric method in order to determine their endogenous concentrations in blood and tissues. However, only traces of bufotenine or DMT were detected in tissues and none in blood. Thus far, these DMIAAs have been found in mammals only in (human) urine, and we expected to find elevated levels in tissues rich in INMT, an enzyme capable of synthesizing these amines from serotonin and tryptamine. A new finding was the detection of large amounts of bufotenine in stools. Although fecal and urinary bufotenine could both, in principle, originate from fecal bacteria, we consider this unlikely. Instead, we propose that bufotenine found in these excretions is synthesized by the cells of the intestinal epithelium and kidney.

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References

- [1] Narasimhachari N, Baumann P, Pak HS, Carpenter WT, Zocchi AF, Hokanson L, et al. Gas chromatographic-mass spectrometric identification of urinary bufotenin and dimethyltryptamine in drug-free chronic schizophrenic patients. *Biol Psychiatry* 1974;8:293–305.
- [2] Räisänen M, Kärkkäinen J. Mass fragmentographic quantification of urinary N,N-dimethyltryptamine and bufotenine. *J Chromatogr* 1979;162:579–84.
- [3] Räisänen MJ. The presence of free and conjugated bufotenin in normal human urine. *Life Sci* 1984;34:2041–5.
- [4] Kärkkäinen J, Räisänen M, Naukkarinen H, Spoov J, Rimón R. Urinary excretion of free bufotenin by psychiatric patients. *Biol Psychiatry* 1988;24:441–6.
- [5] Forsström T, Tuominen J, Kärkkäinen J. Determination of potentially hallucinogenic N-dimethylated indoleamines in human urine by HPLC/ESI-MS-MS. *Scand J Clin Lab Invest* 2001;61:547–56.
- [6] Räisänen MJ, Virkkunen M, Huttunen MO, Furman B, Kärkkäinen J. Increased urinary excretion of bufotenin by violent offenders with paranoid symptoms and family violence. *Lancet* 1984 8404;2:700–1.
- [7] Kärkkäinen J, Räisänen M, Huttunen MO, Kallio E, Naukkarinen H, Virkkunen M. Urinary excretion of bufotenin (N,N-dimethyl-5-hydroxytryptamine) is increased in suspicious violent offenders: a confirmatory study. *Psychiatry Res* 1995;58:145–52.
- [8] Kärkkäinen J, Räisänen M. Nialamide, an MAO inhibitor, increases urinary excretion of endogenously produced bufotenin in man. *Biol Psychiatry* 1992;32:1042–8.
- [9] Axelrod J. The enzymatic N-methylation of serotonin and other amines. *J Pharmacol Exp Ther* 1962;138:28–33.
- [10] Thompson MA, Weinshilboum RM. Rabbit lung indolethylamine N-methyltransferase. cDNA and gene cloning and characterization. *J Biol Chem* 1998;273:34502–10.
- [11] Thompson MA, Moon E, Kim UJ, Xu J, Siciliano MJ, Weinshilboum RM. Human indolethylamine N-methyltransferase: cDNA cloning and expression, gene cloning, and chromosomal localization. *Genomics* 1999;61:285–97.
- [12] Glennon RA. Do classical hallucinogens act as 5-HT₂ agonists or antagonists? *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 1990;5–6:509–17.

- [13] Sanders-Bush E, Oates JA, Bush MT. Metabolism of bufotenine-2'-14C in human volunteers. *Life Sci* 1976;19:1407-11.
- [14] Fuller RW, Snoddy HD, Perry KW. Tissue distribution, metabolism and effects of bufotenine administered to rats. *Neuropharmacology* 1995;34:799-804.
- [15] Barker SA, Littlefield-Chabaud MA, David C. Distribution of the hallucinogens N,N-dimethyltryptamine and 5-methoxy-N,N-dimethyltryptamine in rat brain following intraperitoneal injection: application of a new solid-phase extraction LC-APcI-MS-MS-isotope dilution method. *J Chromatogr B, Biomedical Sci Applications* 2001;751:37-47.
- [16] Walker RW, Ahn HS, Albers-Schonberg G, Mandel LR, Vandenheuvcl WJ. Gas chromatographic-mass spectrometric isotope dilution assay for N,N-dimethyltryptamine in human plasma. *Biochem Med* 1973;8:105-13.
- [17] Angrist B, Gershon S, Sathananthan G, Walker RW, Lopez-Ramos B, Mandel LR, et al. Dimethyltryptamine levels in blood of schizophrenic patients and control subjects. *Psychopharmacology* 1976;47:29-32.