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- Dimethyltryptamine not detected in urine specimens from mediums and non-medium controls
- No difference in bufotenine detection in urine specimens between mediums and non-medium controls
- Mediums reported more anomalous experiences, but similar mental health scores

ACCEPTED MANUSCRIPT

Comparing the detection of endogenous psychedelics in individuals with and without alleged mediumistic experiences

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Abstract

Context: Mediumship is the alleged ability to communicate with deceased personalities. Previous studies have suggested that the endogenous psychotomimetic molecules bufotenine (BT) and dimethyltryptamine (DMT) may play a role in the pathogenesis of psychotic disorders. Distortion of perceptions observed during spiritual experiences could supposedly relate to these substances.

Objective: To compare the presence of BT and DMT in human urine samples between individuals with and without mediumistic experiences.

Methods: All participants (5 from medium's group - MG and 5 from non-medium's group - CG) undertook a single night continuous 6-hour urine pool collection (6:00 to 11:59 PM). Mediums collected urine samples in nights when they reported having experienced mediumistic communication. A sensitive high-performance liquid chromatography-mass spectrometry (HPLC-MS) assay was used. Questionnaires were used to detect common mental disorders symptoms, and to screen and quantify anomalous experiences.

Results: DMT was not detected in any urine specimen tested. The presence of BT detection in urine samples was greater in CG (2/5) than in MG (1/5), with no significant differences ($p > 0.99$). MG reported more anomalous experiences than CG (6.6 ± 0.8 vs. 2.2 ± 1.5 , $p = 0.03$), but there was no difference concerning their mental health.

Conclusion: There were no differences between individuals with and without alleged mediumistic experiences concerning endogenous psychedelics. Both BT and DMT are highly sensitive to metabolism by monoamine oxidase and to N-oxidation, and do not survive in the periphery for long. Alternative strategies should be considered to further investigate the putative role of the endogenous psychedelics pathway for the spiritual experiences.

Keywords

bufotenine; dimethyltryptamine; endogenous; mediumship; high-performance liquid chromatography - mass spectrometry

Introduction

In most Western societies, when a person has anomalous sensorial experiences and behaviors, this is usually interpreted as a sign of mental disease. However, in some cultures, these experiences may be interpreted as a spiritual connection or a “spirit” communication. The prevailing scientific interpretation of the external “communicating spirits” is that they are fragments of the individual’s own inner conflicts.¹ In line with this view, some researchers propose that mediums would be individuals with an intrinsic proclivity to somatization and to dissociation that find in a religious system a way for a positive transformation of their identities, which would consist in a cognitive coping strategy.² Nevertheless, some scholars accept the postmortem survival of consciousness hypothesis as plausible, and do not discard the possibility that some individuals, in altered mental states, could communicate through extrasensory perception with some form of non-local consciousness².

Several traditions emphasize mediumship, such as the case of Spiritism in Brazil. Mediumship can be defined as the alleged ability to communicate with deceased personalities on a regular basis.³ Studies are showing that these experiences have psychotic and dissociative characteristics, but are not associated with mental health problems, since most mediums show good mental health scores, have active working lives and are socially well-adapted.² However, the studies also show that the scores of mental health and control of the anomalous experiences are clearly different in individuals that are beginning to participate in the religion, compared to those that have many years of practice as socially sanctioned mediums, being significantly worse in the former case.²

There are few studies investigating the physiological correlations of mediumistic experiences. Given the similarity of symptoms following the administration of serotonergic hallucinogens and the subjective states of individuals experiencing ecstatic religious occurrences (often leading to depersonalization and out-of-body sensations)^{4,5}, some authors have hypothesized that endogenously generated psychoactive tryptamines could play a role in the distortion of perceptions observed during these occurrences.^{5,6} N,N-dimethyltryptamine (DMT), 5-hydroxy-DMT (bufotenine), and 5-methoxy-DMT (5-MeO-DMT) are three indole alkaloids that possess differing degrees of psychedelic activity and that have been reported as endogenous substances in

humans, in a number of mammals and in amphibians.⁷ In a recent comprehensive review of reports of endogenous psychedelics, Barker et al. (2012) found that there is convincing evidence that DMT and bufotenine are endogenous in humans, whereas the evidence is less compelling for 5-MeO-DMT.⁷

DMT has recently been characterized as an endogenous substrate for sigma 1 receptor, and it possibly acts at trace amine receptors as well. However, the roles of DMT (and bufotenine) in regulating some aspects of human physiology are poorly understood.⁷ Most of research into the presence of these compounds sought their role in mental illness, and there is evidence to suggest that bufotenine urinary levels can be found to be elevated in subjects with psychotic disorder, but no definitive conclusions yet exist.^{7,8} Barker et al. (2012) compiled data from studies examining urine samples for these compounds. For bufotenine, from 1912 individuals investigated (roughly two thirds of them being patients [predominantly diagnosed with schizophrenia] and one third healthy controls), among patients, 71% were positive, whereas among controls, 55% were positive. On the other hand, for DMT, from 861 individuals examined (roughly two thirds being patients and one third healthy controls), among patients, 43% had positive urine samples, and among controls, 64% were positive.⁷ Hence, understanding how psychotic-like experiences such as the mediumistic occurrences may have similarities and differences with mental health disorders can advance this area of research.

Objectives

The present study sought to compare the presence of bufotenine (BT) and dimethyltryptamine (DMT) in human urine samples between individuals with and without mediumistic experiences. Also, questionnaires were used to detect common mental disorders symptoms, and to screen and quantify anomalous experiences. We hypothesized that, in response to the mediumistic experience, greater detection of BT and DMT would be found in mediums than in non-medium control participants. In addition, we expected mediums to have greater scores of anomalous experiences, but similar scores of mental health compared to non-medium control group.

Methods

Participants

Five subjects allegedly capable of mediumistic speaking (mediums group - MG) were recruited from two Spiritist centers with standardized *disobsession* meetings (a type of Spirit release therapy)⁹ and affiliated by the Spiritist Federation of Mato Grosso do Sul, Brazil. Five healthy control subjects with no previous experiences in trance states (control group – CG) were recruited from the staff of a local Medical Clinic, they served as proxies for the average ordinary person. In MG, only individuals participating as mediums in *disobsession* meetings for five years or more were included. Exclusion criteria for both groups were pregnancy, smoking, epilepsy, urinary infection, psychiatric illnesses, chronic diseases as well as current use of psychiatric medicines, antiepileptic or psychoactive drugs (because the presence of these conditions would make the study results more difficult to interpret). People of indigenous origin were excluded for ethical reasons, because of their vulnerable status.

Study design

Having in mind the study *a priori* hypothesis that mediums would present greater detection of BT and DMT, we speculated that this could occur as a state (i.e., greater production of endogenous psychedelics during the mediumistic experience), but also as a trait (i.e., alleged mediums could have higher levels of endogenous psychedelics constitutionally). However, due to the preliminary and exploratory character of the investigation, we chose a study design that we considered able to put more into evidence the differences regarding these substances, assuming they actually had a role in the mediumistic phenomenon. This way, instead of making “within-group comparisons” in mediums, we elected to compare the detection of substances in urine samples from mediums after the trance, with the detection in samples from non-medium controls in normal condition. Data from all participants were collected in the same week, in June 2017. A single night continuous 6-hour urine pool collection (6:00 to 11:59 PM) was taken from each participant. Participants in the MG collected the urine

samples after regular *disobsession* meeting nights, when they reported having experienced at least one mediumistic communication. Participants in the CG collected the urine samples in their homes, in nights in which they did not exercise.

All participants received labeled sterile flasks for use in a continuous 6-hour urine pool collection, with the direction to collect all micturition from 6:00 to 11:59 PM. The samples were kept at 4 °C until the end of the sampling when flasks containing urine were transferred (in the dark, in a refrigerated bag) to the lab where the samples were centrifuged (3900 rpm, 15 min) and aliquoted in 1 mL final volume, and then stored at -20 °C (*Célula Diagnósticos Citológicos & Análises Clínicas* - Campo Grande/ MS).

Self-reported Instruments

Participants answered the following questionnaires prior to the specimen collection night:

- The Anomalous Experiences Inventory¹⁰ (Menezes Jr., Alminhana & Moreira-Almeida, 2012) is a fourteen-item multiple-choice instrument, in Portuguese language, where participants choose which anomalous experiences (AE) have already happened to them (e.g., apparitional experiences, spiritual hearing, out-of-body experiences, psychography, etc). We chose this instrument because it seems well-suited to screen for and to quantify mediumistic experiences, and because it had been successfully used before in this context.

- Mental health was assessed by the "Self-Report Psychiatric Screening Questionnaire" [SRQ- 20] validated in Portuguese.¹¹ This is a 20-item questionnaire formulated to detect common mental disorders in primary care. Seven or more positive (yes) answers suggest a mental disorder. We chose this instrument because it is widely used in research, easy to apply and had also been successfully used in the context of alleged mediumship.

Implementation of method to detect dimethyltryptamine and bufotenine in human urine

The method to detect bufotenine and DMT in urine was implemented at the Lychnoflora Research and Development of Natural Products (Ribeirão Preto/ SP), using a high-performance liquid chromatography-mass spectrometry (HPLC-MS) method as previously described.¹² The experiments were carried out on a Liquid Chromatography equipment UFLC Shimadzu (Kyoto, Japan), using a Phenomenex SPE C18, Strata (55 μ m, 70Å) column (Aschaffenburg, Germany); the LC system was coupled to a 3200 QTrap triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada).

The internal standard working solutions for HPLC-MS method were prepared with analytical samples of bufotenine and *N,N*-dimethyltryptamine (DMT) from the Institute of Chemistry of the Brasília University/ UnB - Brasília/ DF. At this Institute, Moreira and coworkers (2015) have previously isolated the alkaloid bufotenine from the seeds of Brazilian native plant of the genus *Anadenanthera peregrina* (“angico”) in high purity, by using a modification of Stromberg method.¹³ DMT was prepared by reductive alkylation from the commercially available tryptamine (Aldrich) according to Qu et al. (2011).¹⁴

First, the purity of the internal standard solutions was proofed with a Liquid Chromatography with Diode Array Detection (LC-DAD) method. Subsequently, based on the internal standard solutions, the “Multiple Reaction Monitoring” (MRM) method was undertaken (targeted mass spectrometry), and the ion transitions to be monitored for the screening of the specified peptides in the urine samples were defined.

Urine sampling and analysis

↳ The urine samples were submitted to solid phase extraction according to a previously described method.¹² Subsequently, the samples were analysed via HPLC-MS. In the chromatograms, signal-to-noise ratios (S/N) were calculated for the retention times in the target ion transitions corresponding to bufotenine and DMT. The analyte was considered present in the sample when the signal-to-noise ratio was greater than 3,0. The strategy adopted was to utilize the

present method as a preliminary categorical analysis and, according to the achieved results, consider progressing to a further research stage when validation parameters could be obtained allowing precise quantification of the compounds.

Beyond the analyses of urine samples of participants, the same procedure was applied in a quality control synthetic urine sample (PNCQ - Rio de Janeiro/ RJ). Results were expressed as “presence” or “absence” of bufotenine and DMT. Laboratory personnel were blinded to the group status of the study participants.

Statistical analysis

The data collected were entered to the Graph Pad InStat Software, version 3.06. For the between-group comparisons, the following tests were used: Fisher's exact test (categorical variables); unpaired t test (continuous variables that were normally distributed); and Mann-Whitney test (continuous variables that were not normally distributed). A two-tailed p value ≤ 0.05 was considered statistically significant and the confidence interval was set at 95%. Values are reported as means \pm standard error of means (SEM).

Ethical issues

The study was approved by the Institutional Review Board of the Federal University of Mato Grosso do Sul (number: 67166917.4.0000.0021). The study was carried out in accordance with the International Ethical Guidelines and Declaration of Helsinki.

Results

A total of 10 participants (5 from the MG and 5 from the CG) participated in the study. There were no differences in participants' age (MG: 55.2 ± 7.4 vs. CG: 41.0 ± 0.9 years, $p = 0.092$), and gender (MG: 4 females vs. CG: 3 females, $p > 0.999$). Subjects in the MG reported a significantly higher number of anomalous experiences than the subjects in the CG (6.6 ± 0.8 vs. 2.2 ± 1.5 , $p =$

0.032). However, no differences in subjects' mental health were found between groups (mean SRQ scores MG: 2.0 ± 0.5 vs CG: 2.2 ± 0.7 , $p > 0.999$), and all participants from both groups scored lower than the cut-off of 7 positive answers.

DMT was not detected in any of the urine specimens tested. Table 1 shows the results of bufotenine detection in the urine samples via liquid chromatography – mass spectrometry (LC-MS) analysis and no between-group difference was noted for this parameter ($p > 0.999$).

Table 1 Results of bufotenine detection in urine samples analysed via liquid chromatography – mass spectrometry (LC-MS) analysis – (Multiple Reactions Monitoring – MRM), using signal-to-noise ratios (S/N), between groups.

Bufotenine detection	Group		Statistic (Fisher's Exact Test)
	Mediums (n=5)	Controls (n=5)	
Yes	1 (20%)	2 (40%)	$p > 0.999$
No	4 (80%)	3 (60%)	

Figure 1 shows LC-MS (MRM) spectra for the evaluation of bufotenine in some urine samples. All urine samples that screened positive were from female participants. Neither bufotenine nor DMT were detected in the quality control synthetic urine sample.

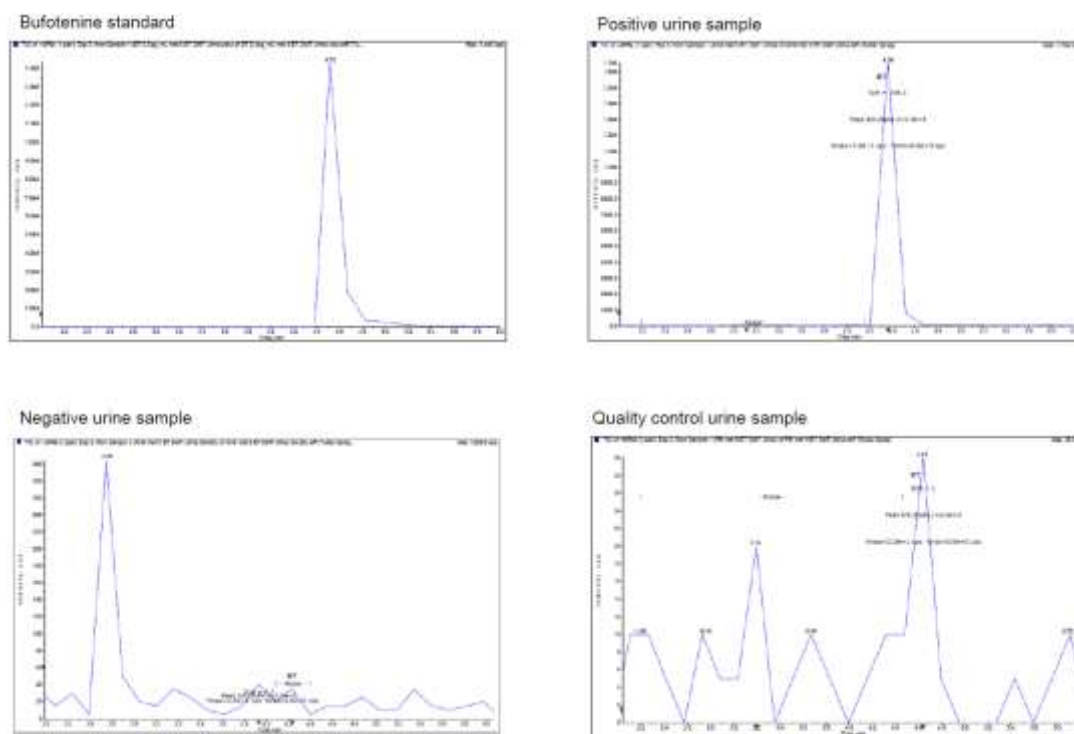


Fig.1. LC-MS (MRM) spectra for the evaluation of bufotenine (BT) in urine samples (milli absorbance units [m AU]/ minute)

Discussion

The present preliminary study found no differences on the presence of bufotenine in individuals with and without mediumistic experiences and DMT was not detected in any of the urine specimens tested. These findings do not support our hypothesis that a greater detection of bufotenine and DMT would be found in the periphery (urine samples) of mediums compared to non-medium control participants. The previous findings of higher levels of bufotenine in urine samples of psychotic patients were not corroborated in the present preliminary investigation on a psychotholic-like experience (mediumship). The absence of DMT in all urine specimens tested was very surprising as previous studies have demonstrated positive results in roughly half of individuals tested, whether psychiatric patients or healthy controls.

With respect to the questionnaire measures, our *a priori* hypothesis was confirmed. Mediums had greater scores of anomalous experiences, but similar scores of mental health compared to non-medium controls. This is line with the

literature on the field, especially considering that our sample consisted of experienced mediums (five or more years of practice).

We can present the following reasons to explain our findings. First, some authors hypothesize that endogenous hallucinogens will have higher levels in the central nervous system of mediums. Although we used sensitive exact mass/LC/MS instruments, we tried to detect peripheral measures and, since both DMT and bufotenine are highly sensitive to metabolism by monoamine oxidase (MAO) and to N-oxidation (N-oxide), they usually do not survive in the periphery for long. Second, some cells of the intestinal epithelium or the kidney may also synthesize these substances¹⁵, which may have influenced our results. Third, the small sample size certainly resulted in low statistical power and only remarkable differences between groups would be detected. However, since there was no statistically significant tendency between groups, we consider that the results so far do not point to a favorable cost-benefit ratio of continuing these analyses.

Conclusions

Based on the results of this preliminary study, we discourage measurement of DMT and bufotenine in urine samples for similar purpose. Further investigations on the putative role of the endogenous hallucinogens pathway for the spiritual experiences should examine blood samples, or maybe focus on alternative strategies. Radioligand studies accessing the variability in 5-HT_{2A} receptor density in the CNS and studies of genetic polymorphisms of these receptors represent other promising approaches to address this topic.

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Conflicts of interest

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