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Elevated urine levels of bufotenine in patients with autistic spectrum disorders and schizophrenia

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Abstract

OBJECTIVE: Previous studies have suggested that the endogenous psychotomimetic molecule bufotenine (N-N-dimethyl-5-idroxytryptamine) may play a role in the pathogenesis of severe mental disorders. The potential association of bufotenine with the clinical features of autism and schizophrenia is not entirely understood. In this study, we measured urinary levels of bufotenine in subjects with autistic spectrum disorder (ASD), schizophrenia and healthy comparison subjects free of psychiatric symptoms. We also sought to assess whether urine concentrations of this molecule may be associated with the clinical characteristics of psychiatric patients.

DESIGN: Urine bufotenine levels were measured using a high-performance liquid chromatography-mass spectrometry (HPLC-MS) assay in young adults with severe ASD (n=15), patients with schizophrenia (n=15), and healthy control subjects (n=18). The Vineland Adaptive Behavior Scale was used to measure adaptive behaviors in ASD individuals. The Brief Psychiatric Rating Scale (BPRS) was used for patients with schizophrenia.

RESULTS: Urine bufotenine levels were significantly higher in ASD subjects ($3.30 \pm 0.49 \mu\text{g/L}$, $p < 0.05$) and patients with schizophrenia ($4.39 \pm 0.43 \mu\text{g/L}$, $p < 0.001$) compared with controls

($1.53 \pm 0.30 \mu\text{g/L}$). Among patients with ASD, there was a significant positive correlation between urine bufotenine and hyperactivity scores on the Vineland Adaptive Behavior Scale ($r = 0.479$, $p < 0.05$). No other associations were detected.

CONCLUSIONS: Our results indicate that elevated urine levels of the endogenous psychotomimetic molecule bufotenine may play a role in ASD and schizophrenia, and can be correlated with hyperactivity scores in autism.

INTRODUCTION

Previous studies have suggested that endogenous psychotomimetic molecules may be involved in the pathogenesis of major psychiatric disorders (Osmond & Smythies 1952; Smythies 1983; Ciprian-Ollivier *et al.* 1988). Bufotenine (5-hydroxy-N,N-dimethyltryptamine) is a tryptamine alkaloid derived from the double methylation of serotonin commonly found in a number of mammals and in several amphibian groups (Takeda 1994). There is evidence to suggest that bufotenine has potent psychotropic actions in humans, probably due to its similar physiological and structural features to LSD on the 5HT₂ receptor (McBride 2000). Interestingly, an endogenous production of bufotenine has been previously reported in patients with several psychiatric disease, and urine levels of this molecule have been found to be elevated in subjects with schizophrenia in numerous (Fischer & Spatz 1968; Räisänen *et al.* 1984; Fischer *et al.* 1971; Narasimhachari & Himwich 1972; Cottrell *et al.* 1977) but not all (Perry *et al.* 1966; Siegel 1956; Rodnight 1956; Wyatt *et al.* 1973) studies. Of interest is also the observation that bufotenine has been reported to be higher in the urine of patients with autistic spectrum disorder (ASD) (Piggott 1979; Himwich *et al.* 1972) as well as their parents (Narasimhachari & Himwich 1975). Notably, a previous study has detected bufotenine at significant amounts in 32/47 autistic patients and in 18/18 patients with mental retardation, whereas it was found in only 2 out of 200 controls (Takeda *et al.* 1995). Urinary excretion of bufotenine has been also found to be higher in drug-free violent offenders, and levels of this molecule have been found to be positively associated with suspiciousness and aggression and negatively with socialization scores (Kärkkäinen *et al.* 1995; Räisänen *et al.* 1984). In this study, we assessed urine levels of bufotenine in subjects with autistic spectrum disorder (ASD), schizophrenia and healthy comparison subjects free of psychiatric symptoms. We also sought to investigate whether urine bufotenine levels may be associated with the clinical characteristics of psychiatric patients.

MATERIALS AND METHODS

Subjects

We measured urine bufotenine levels in the following three groups of subjects: adults with severe ASD (n = 15; 13 males and 2 females, mean age: 31.0 ± 7.5 years), patients with schizophrenia (n = 15; 10 males and 5 females, mean age: 32.7 ± 8.6 years), and normal control subjects (n = 18; 15 males and 3 females, mean age 31.9 ± 8.0 years). Patients with ASD were recruited from a single farm community center specifically designed for individuals with autism (Cascina Rossago, San Ponso Semola, Pavia, Italy). The diagnosis of ASD was confirmed in all participants jointly by two independent psychiatrists specializing in autism who made the diagnosis according to the guidelines of the Structured

Clinical Interview for Axis I DSM-IV Disorders, Clinical Version (First *et al.* 1996). All patients in the present study scored more than 30 on the Childhood Autism Rating Scale (CARS) (Schopler *et al.* 1988), the standard threshold used to distinguish autism. The Vineland Adaptive Behavior Scale (Sparrow *et al.* 1984) was used to measure adaptive behaviors in ASD individuals.

Patients with schizophrenia were recruited from the Department of Psychiatry at the Pavia University School of Medicine and from Outpatients Departments from which we receive referrals. Each patient was given a diagnostic assessment by an experienced psychiatrist based on the Structured Clinical Interview for DSM-IV (American Psychiatric Association 2005). The psychopathological status of patients with schizophrenia was assessed using the Brief Psychiatric Rating Scale (BPRS) (Ventura *et al.* 2000). All psychiatric patients were either medication-naïve (first-onset) or medication-free for at least four months. Control subjects were recruited from healthy blood donors or volunteers who had helped in other studies within our institutions. Comparison subjects were drawn from the same geographical area as our patient group, aiming to recover the basic demographics of the regions from which the patients were recruited. All controls had no past or present history of any psychiatric disease and none of them had ever taken medications for psychiatric conditions. Additionally, subjects with axis-I diagnosis of first degree relatives were not included in this group.

The study was approved by the local ethics committee in accordance to the Helsinki Declaration and written informed consent was obtained from all participants or legal guardians.

Laboratory methods

Specimens of the middle urinary flow were collected using a standard sterile urine container, transported in the dark in a refrigerated bag and frozen at -40°C within 3 hours of collection. Bufotenine concentrations in urine were determined using a high-performance liquid chromatography-mass spectrometry (HPLC-MS) method as previously described (Kärkkäinen *et al.* 2005), with slight modifications. Urine bufotenine was determined in a quality control sample with within-series and between-series coefficients of variation of 3.8 and 4.6%, respectively. Since laboratory personnel were blinded to the clinical status of the study participants, any possible measurement error was likely to be non-differential.

Data analysis

Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov statistics. Since all variables were normally distributed only parametric statistics were used. Continuous data are expressed as means ± standard deviations. Comparison of categorical variables was generated by the χ^2 test. For three group comparisons of quantitative variables, a one-way

Tab. 1. General characteristics of the study participants.

	ASD patients (n=15)	Schizophrenia (n=15)	Controls (n=18)	p-value
Age, years	31.0±7.5	32.7±8.6	31.9±8.0	0.59
Males/females	13/2	10/5	15/3	0.35
Body mass index, kg/m ²	23.9±3.3	24.7±3.6	24.6±3.4	0.47
Glucose, mmol/L	4.6±0.7	4.5±0.6	4.7±0.7	0.77
Creatinine, mg/dL	0.82±0.14	0.84±0.18	0.83±0.19	0.61
Total cholesterol, mmol/L	4.6±0.7	4.8±0.8	4.7±0.7	0.69
LDL cholesterol, mmol/L	2.8±0.8	2.9±1.0	3.1±1.0	0.30
HDL cholesterol, mmol/L	1.4±0.4	1.5±0.4	1.4±0.5	0.89
Triglycerides, mmol/L	1.6±0.9	1.6±1.0	1.5±1.1	0.67
Serum sodium, mmol/L	141.9±3.2	142.0±3.5	140.8±3.0	0.91
Serum potassium, mmol/L	4.1±0.5	4.0±0.5	4.0±0.6	0.84
Systolic blood pressure, mmHg	128±9	130±10	126±7	0.21
Diastolic blood pressure, mmHg	82±7	84±11	81±6	0.18
Urine bufotenine, µg/L	3.30±0.49	4.39±0.43	1.53±0.30	< 0.001

p-values are calculated by means of ANOVA or χ^2 test, as appropriate.

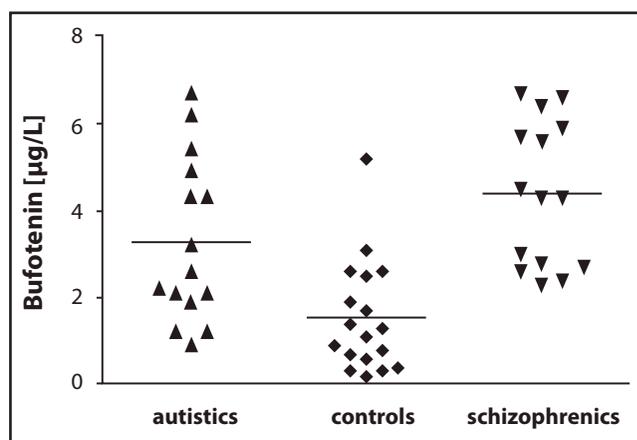


Fig. 1. Scatter diagram for urine bufotenine in the three study groups (ASD, controls, and schizophrenia). Horizontal lines across the scatter diagram represent mean values.

analysis of variance (ANOVA) with post-hoc Dunn's testing was performed. Correlations among variables were computed with the use of Pearson's correlation coefficients. All statistical analyses were carried out using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 4.0 (GraphPad Software Inc., San Diego, CA, USA). A two-tailed p -value <0.05 was considered statistically significant.

RESULTS

The general characteristics of the study participants are depicted in Table 1. No significant differences in demographic and clinical parameters were

detected among different groups. Urine bufotenine levels were significantly higher in the ASD group (3.30 ± 0.49 µg/L, $p < 0.05$) and in patients with schizophrenia (4.39 ± 0.43 µg/L, $p < 0.001$) compared with controls (1.53 ± 0.30 µg/L, Figure 1). Although there was a trend towards higher levels of urine bufotenine in patients with schizophrenia than in ASD, this difference failed to reach the statistical significance threshold. Among patients with ASD, there was a significant positive correlation between urine bufotenine and hyperactivity scores on the Vineland Adaptive Behavior Scales ($r = 0.479$, $p < 0.05$). No significant correlations were found between BPRS scores and urine bufotenine levels in patients with schizophrenia (data not shown).

DISCUSSION

The present study provides evidence of a significant elevation of urine bufotenine levels in patients with ASD and schizophrenia compared with a matched control population. Additionally, concentrations of bufotenine in urine were found to be positively associated with hyperactivity scores on the Vineland Adaptive Behavior Scales in ASD subjects. Although no correlation was evident between urinary levels of this molecule and the severity of schizophrenia symptoms, our data point to a subtle but definite role of this endogenous psychotomimetic molecule in ASD and schizophrenia.

Subtle alterations in serotonin metabolism have been suggested to occur and play a role in the pathogenesis of schizophrenia and autism. Bufotenine has been found to act as a potent endogenous hallucinogenic factor, with an activity similar to LSD at the purported hallucinogenic serotonin receptors, 5HT_{2A} and

5HT2C (McBride 2000). Although some reports have reported detectable levels of urinary bufotenine in several groups of patients with major psychiatric illnesses (Fischer & Spatz 1968; Räisänen *et al.* 1984; Fischer *et al.* 1971; Narasimhachari & Himwich 1972; Cottrell *et al.* 1977), other authors were unable to confirm the presence of this molecule (Perry *et al.* 1966; Siegel 1956; Rodnight 1956; Wyatt *et al.* 1973). One of the reasons for such discrepancy may be the lack of standardization of methods for the determination of urinary bufotenine levels and different ways in which urine samples are handled. In this study, we used an HPLC-MS method to detect urine bufotenine as previously described (Kärkkäinen *et al.* 2005). Of note, we were able to confirm the presence of a detectable amounts of bufotenine not only in patients with psychiatric disorders, but also in controls. This result is in keeping with those obtained by Kärkkäinen and coworkers (Kärkkäinen *et al.* 2005), but conflict with those obtained by earlier studies (Perry *et al.* 1966; Siegel 1956; Rodnight 1956; Wyatt *et al.* 1973). An improved sensitivity of the laboratory techniques used to assess urine bufotenine may account for such findings.

In our study, we found that urine bufotenine was significantly higher in patients with ASD and schizophrenia compared with controls. These findings were in line with those obtained by previous studies in schizophrenia and autism (Fischer & Spatz 1968; Räisänen 1984; Fischer *et al.* 1971; Narasimhachari & Himwich 1972; Cottrell *et al.* 1977, Himwich *et al.* 1972; Piggott 1979). However, previous studies did not clarify whether urine bufotenine might be associated with the clinical features of major psychiatric illnesses. In our report, a significant positive correlation between hyperactivity scores and urine bufotenine levels was evident in patients with ASD. It is feasible to hypothesize that increased levels of bufotenin in autistic subjects with hyperactivity can be a biological correlate of their behavior or – more speculatively – be a causal factor. Intriguingly, our data parallel those of Kärkkäinen *et al.* (1995) who showed that violent offenders with paranoid personality traits have higher urinary levels of bufotenine than other violent offenders. Additionally, Räisänen *et al.* have suggested that urinary excretion of bufotenine is increased in violent offenders with paranoid symptoms and family violence (Räisänen *et al.* 1984). Although these findings may prompt intriguing hypotheses on the possible association of bufotenine levels in urine and aberrant behaviors, further investigations are needed in larger sample size to draw a more definite conclusion on this issue.

Caveats of this study merit consideration. The chief limitations of our report are the small sample size and its cross-sectional design. We thus observed associations, not prediction or causation. Therefore, it remains to be established whether increased bufotenine level observed in autism is a cause or a consequence of disease. In addition, we limited our analysis to bufotenine levels as measured in the urine. In light of this limita-

tion, it remains to be established whether the increase of peripheral bufotenine observed in autism may be paralleled by similar changes in the central nervous system. In this regard, it has been previously suggested that the CNS is a favored site for the accumulation of bufotenine, probably because of the lipophilic properties of this substance and the slow catabolism of methylated indolamines in the brain (Kärkkäinen *et al.* 2005). The presence of a significantly higher urinary bufotenine concentration is another evidence of the complex serotonergic alterations occurring in autism and schizophrenia, which probably includes an abnormal methylation of this neurotransmitter.

In summary, our results indicate that elevated urine bufotenine levels may play a role in schizophrenia and ASD and may be correlated with hyperactivity scores in autism.

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