NeuroReport 6, 2378–2380 (1995)

Bufotenine reconsidered as a diagnostic indicator of psychiatric disorders

Naokuni Takeda,[1,CA] Ryoichi Ikeda,[2] Kimitaka Ohba[3] and Masanori Kondo[4]

[1]Department of Biotechnology, Research and Development Center, COSMO Research Institute, Satte, Saitama, 340-01, Japan; [2]Department of Neuropsychiatry, Tokyo Medical College, Nish-Shinjuku, Tokyo; [3]Oshima Colony Center, Kami-Iso, Hokkaido; [4]Tokyo Medical College, Kasumigaura Hospital, Ami-machi, Inashiki, Ibaraki, Japan

[CA]Corresponding Author

WE have analyzed products of the serotonin-degradative pathway, in which both *N*-methylserotonin and bufotenine are formed in urine specimens of products with psychiatric disorders by three-dimensional HPLC with electrochemical detection. Bufotenine was detected in urine from all autistic patients with mental retardation and epilepsy ($n = 18$) and many autistic patients (32/47) with mental retardation. Bufotenine was detected in the urine of 15 of 18 patients with depression. Thirteen of 15 schizophrenic patients were also positive for bufotenine. *N*-methylserotonin was also detected in some cases of each disorder. Only two of 200 urine specimens from healthy controls were positive for bufotenine. Thus, the presence and levels of bufotenine might be useful and important markers of some psychiatric disorders.

Key words: Bufotenine; *N*-methylserotonin; Psychiatric disorder; Methylation theory

Introduction

The first evidence, obtained by paper chromatography, that urine from schizophrenic patients contains a bufotenine-like (BUTN-like) substance, in contrast to the urine of healthy controls, was reported by Fisher *et al.*[1] In the 1960s, there were many attempts to determine whether BUTN or a BUTN-like substance was present in urine from normal controls or schizophrenic patients.[2] At that time, however, methods for detection of BUTN were too insensitive to allow measurements of minute quantities. In spite of the positive results obtained with urine from schizophrenics, little further progress was made until the Kärkkhinen and coworkers reported the detection of BUTN by gas chromatography-mass spectrometry (GC-MS) in 1988.[3] Now, three-dimensional high-performance liquid chromatography with electrochemical detection (HPLC with ECD), allows indoleamines including BUTN and also catecholamines derived from tryptophan and tyrosine, as well as their metabolites, to be easily and simultaneously detected and quantitated with a high degree of accuracy.[4–9]

BUTN (5-hydroxy-*N*,*N*-dimethylserotonin) is a dimethylated derivative of serotonin and is known as a hallucinogen. It is found in the nematocytes of sea anemones and in the epidermal secretions of some toads, as well as in seeds of some species of *Anadenathera* and *Piptadenia* and in certain mushrooms in the genus *Amanita* species that induce hallucinations upon ingestion. The normal metabolic pathways from serotonin are as follows: serotonin → 5-hydroxyindoleacetic acid (5-HIAA) and serotonin → *N*-acetylserotonin → melatonin (MEL). The serotonin-degradative pathway examined in the present study is as follows: serotonin → *N*-methylserotonin (N-MET) → BUTN. Thus serotonin-degradative pathway, which does not involve 5-HIAA and MEL, is an unusual route that is associated with the production of venoms or hallucinogens and is not associated with normal homeostasis in animals.

In a phylogenetic analysis of the presence of unusual metabolites of serotonin in the nevous system. Takeda happened to find high levels of BUTN in the brain of *Bufo bufo japonicus*,[8] as well as the intermediate metabolite, N-MET, in other species such as *Rana japonica*[5] and *Hydra magnipapillata*.[9] N-MET is a intermediate metabolite of serotonin and can be metabolized to BUTN in the presence of inhibitors of monoamine oxidase, such as nialamide, in *Rana*.[5] An enzyme that can methylate serotonin to generate BUTN has been found in the human brain.[10]

At the present time, there are no chemical indicators that can be used to diagnose the psychiatric abnormalities in humans. Therefore, the unusual metabolism of serotonin in the brain of *Bufo* reminded us of the postulated relationship between the metabolism of serotonin and human psychiatric disorders that originate from functional diseases of the central nervous system. As a consequence BUTN was re-identified by us as a candidate for a diagnostic

indicator of psychiatric disorders from the view point of comparative biology. In humans, bufotenine has been shown to disappear rapidly from the blood and to appear in the urine.[11]

In the present study, therefore, we examine whether or not BUTN or N-MET could be detected in urine specimens from patients with various psychiatric disorders.

Materials and Methods

Urine specimens were collected from patients who were diagnosed as having psychiatric disorders by reference to DSM-IIIR (American Psychiatric Association, 1987). In our hospitals. The majority of patients studies were inpatients and a few patients were outpatients. The severity of each disorder and the duration of hospitalization varied. Patients had been variously treated with drugs such as imipramine as an antidepressant, carbamazepine, phenobarbital and sodium valproate as antiepileptic drugs, and haloperidol and lorazepam as tranquilizers. Seven patients diagnosed as suffering from depression were free from clinically administered drugs at the time of urine collection. All the patients in hospital had eaten regular hospital food, and the absence of any influence of diet on the urinary excretion of BUTN has been demonstrated.[12] Control urine specimens were collected randomly from male members of the staff at Cosmo Research Institute who were normally engaged in research studies. None had taken any drugs at the time of urine collection during a routine medical examination. As a general rule, samples were the first urine specimens passed in the morning.

For analytic studies, tryptophan, serotonin, N-MET, BUTN, 5-hydroxyindoleacetic acid, *N*-acetylserotonin and MEL were quantitated simultaneously by a three-dimensional HPLC system (Coulometric Electrode Array System;[13] ESA Inc., Bedford, MA, USA). The system consists of a gradient-coupled HPLC system and 16 high-sensitivity coulometric electrochemical detectors linked to a compatible computer. A reverse-phase C_{18} column (4.6 mm i.d. × 150 mm; NBS, Tokyo, Japan) was used. Purity of samples in peaks was determined from a 'ratio accuracy'.[8] The ratio accuracy compares ratios of peak height across electrodes for compounds in a mixture of standards with those in the sample. A ratio accuracy of over 90% was taken as being sufficiently high to allow an identification to be made. After centrifugation of urine at 6,000*g* for 10 min. 80 μl of each urine specimen were injected directly onto the column. A part of each sample was also assayed for creatinine with a Creatinine Measuring Kit (Creatinine II-HA Test; Wako, Tokyo, Japan) and an autoanalyzer (Moel 7160; Hitachi, Tokyo, Japan).[14]

Results and Discussion

BUTN was detected in the urine from all autistic patients with mental retardation and epilepsy (n = 18). Seven of the 18 patients were positive for N-MET. Among urine specimens from 47 autistic patients with mental retardation, 32 were positive for BUTN and 8 were positive for N-MET. In cases of infantile autism, Takeda detected only BUTN and not N-MET in urine specimens.[8] BUTN was detected in all of 6 cases of mental retardation with epilepsy or cerebral palsy, and N-MET was found in 4 of the 6 cases. Six patients with epilepsy were all positive for BUTN and 5 of them were positive for N-MET. In 18 patients with depression, BUTN was detected in 15 and N-MET in 17, of whom 7 were new patients who were positive for both BUTN and N-MET. Of 15 schizophrenic patients, 13 were positive for BUTN and 7 were positive for N-MET. In addition, these compounds were also found in the urine of other psychiatric patients, although sample size was small. These included 4 patients with Alzheimer's disease, BUTN was detected in 3 and N-MET in 4. In 4 patients with neurotic insomnia, BUTN was detected in 3 and N-MET was detected in all 4. N-MET but not BUTN was detected in 4 patients with hypochondriasis. Urine from patients with other disorders, such as liver disease and thyroidism, was negative for both compounds. Although, urine specimens from almost all healthy controls were negative, two of 200 urine specimens were positive for BUTN. Characteristically, levels of serotonin, N-MET and BUTN were all high in patients with schizophrenia and low in patients with autism, as compared with those in controls. The main results are summarized in Table 1. These results indicate that the degradative pathway from serotonin to BUTN was associated with each of the various psychiatric disorders. Metabolites other than BUTN and N-MET were not detected, with the exception of 5-HIAA, which was present at normal levels. The activity of the BUTN pathway was high in patients with schizophrenia, depression and epilepsy, in that order, and it was low in patients with autism. The various patients that we examined are still hospitalized, and it is now necessary to examine whether the severity of their symptoms tends to be proportional to the levels of BUTN or N-MET in their urine. Furthermore, it is necessary to trace the dynamics of BUTN and N-MET after treatment with psychoactive drugs.

Since the brain of *Bufo bufo japonicus* has been suggested to provide a useful pharmacological model for BUTN metabolism,[8] many drugs have been tested for their ability to reduce levels of BUTN in the brain of this toad. In *Bufo*, *p*-chlorophenylalanine and ritanserin have been shown to reduce brain levels of BUTN.[6] Our observations encourage the develop-

Table 1. Levels of BUTN and related compounds in urine specimens of patients with various psychiatric disorders

Compounds Disorders	Serotonin	N-MET ng/mg Creatinine (no. of patients)	BUTN	age (years)
Autism				
(a) with mental retardation ($n = 47$)	10.8 ± 4.8 (47)	3.0 ± 3.3 (8)	3.3 ± 1.5 (32)	20 ± 4
(b) with epilepsy and mental retardation ($n = 18$)	12.1 ± 9.6 (18)	2.5 ± 1.7 (7)	3.6 ± 4.3 (18)	22 ± 3
Mental retardation with epilepsy or cerebral palsy ($n = 6$)	7.2 ± 3.9 (6)	2.0 ± 0.8 (3)	2.5 ± 2.0 (6)	21 ± 4
Epilepsy ($n = 6$)	118.7 ± 78.0 (6)	102.3 ± 85.9 (4)	13.7 ± 15.6 (5)	26 ± 5
Depression ($n = 18$)	94.7 ± 116.3 (18)	43.8 ± 48.4 (17)	46.0 ± 48.2 (15)	26 ± 14
Schizophrenia ($n = 15$)	446.4 ± 693.8 (15)	16.4 ± 377.8 (7)	287.9 ± 487.6 (13)	32 ± 11
Non-psychiatric disorders ($n = 30$)	69.4 ± 97.7 (30)	– (0)	– (0)	34 ± 14
Healthy controls ($n = 200$)	84.6 ± 26.8 (200)	– (0)	10.9 ± 8.6 (2)	37 ± 9

ment of new drugs that reduce the levels of these compounds. The old 'methylation theory' of mental disorders seems to have some non-sequential validity.[15]

Several researchers have found BUTN at low concentrations in urine of apparently normal controls.[3,12] However, although the limit of detection of authentic BUTN is about 50 ng ml^{-1} with the present apparatus, we only detected it in tw of 200 healthy normal individuals. One reason for this discrepancy might be radial differences among subjects who were analysed, since our subjects were exclusively Japanese. BUTN has been shown to be capable of producing a schizophrenia-like syndrome. When administered to humans as a hallucinogen or when present in excess in the body, BUTN can cause psychotic behaviour.[16] Recent research has shown that BUTN has an effect on serotonin neurones similar to that of *p*-chloroamphetamine in rats.[17]

At present, we have no chemical markers to assist in the diagnosis of psychiatric disorders. Therefore, we propose here that levels of BUTN in urine be determined in large-scale primary screenings of psychiatriac patients in an attempt to estalish whether BUTN is a useful marker for the diagnosis of psychiatric disorders.

Conclusion

The methylation theory of psychiatric disorders suggested that the human body is capable of producing toxic methylated substances that induce psychotic behaviour. We re-examined it from the view point of comparative biology. The main compounds in the normal metabolism of serotonin and in a degradative pathway to BUTN were analysed simultaneously in urine specimens from psychiatric patients by three-dimensional HPLC with ECD. We focused our attention on BUTN, which is a methylated derivative of serotonin that is known as a hallucinogen, and on N-MET. BUTN was detected in urine samples from all autistic patients with mental retardation and epilepsy and in the majority of autistic patients with mental retardation. BUTN was also detected in patients with depression and schizophrenia. N-MET was also detected in some patients with each psychiatric disorder. In patients with non-psychiatric disorders, neither BUTN or N-MET was detected, and only few urine specimens from healthy controls were positive for BUTN. Therefore, the presence and levels of BUTN suggest a possible route towards the chemical diagnosis of certain psychiatric disorders.

References

1. Fisher E, Fernandez Lagravere TA, Vazquez AJ *et al. J Nervous Mental Disease* **133,** 441–444 (1961).
2. Wyatt RJ, Termini Ba and Davis JM. *Schizophr Bull* **4,** 10–66 (1971).
3. Khrkkäinen J, Räisänen M, Naukkarinen H *et al. Biol Psychiatry* **24,** 441–446 (1988).
4. Takeda N. *Soc for Neurosci* **19,** 1171 (1993).
5. Takeda N. *Soc for Neurosci* **20,** 1165 (1994).
6. Takeda N. *Soc for Neurosci* **21,** 1370 (1995).
7. Takeda N. *Amer Zool* **35,** 741 (1992).
8. Takeda N. *Comp Biochem Physiol* **107C,** 275–281 (1994).
9. Takeda N and Svendsen CN. *J Hydrobiol* **216/217,** 549–554 (1991).
10. Mandell AJ and Morgan M. *Nature* **230,** 85–87 (1971).
11. McLeod WR and Sitaram BR. *Acta Psychiatr Scand* **72,** 447–450 (1985).
12. Sireix DW and Marini FA. *Biol Psychiatry* **1,** 189–191 (1969).
13. Matson WR, Lanmglais P, Volicer L *et al. Clin Chem* **30,** 1477–1488 (1984).
14. Takeda N. *Comp Biochem Physiol* **105,** 373–377 (1993).
15. Baldessarini RJ, Stramentinoli G and Lipinski JF. *Arch Gen Psychiatry* **36,** 303–307 (1979).
16. Fabing HD and Hawkins JR. *Science* **123,** 886–887 (1956).
17. Monroe PJ, Smith DL, Williams GM *et al. Biogenic Amines* **10,** 273–284 (1994).

Received 23 June 1995; resubmitted 28 July 1995; accepted 23 August 1995