Research article

Biomedical Chromatography

Received: 20 March 2013, Revised: 20 May 2013, Accepted: 23 May 2013 Published online in Wiley Online Library: 23 July 2013

(wileyonlinelibrary.com) DOI 10.1002/bmc.2981

LC/MS/MS analysis of the endogenous dimethyltryptamine hallucinogens, their precursors, and major metabolites in rat pineal gland microdialysate

Steven A. Barker[a]*, Jimo Borjigin[b], Izabela Lomnicka[a] and Rick Strassman[c]

ABSTRACT: We report a qualitative liquid chromatography–tandem mass spectrometry (LC/MS/MS) method for the simultaneous analysis of the three known *N,N*-dimethyltryptamine endogenous hallucinogens, their precursors and metabolites, as well as melatonin and its metabolic precursors. The method was characterized using artificial cerebrospinal fluid (aCSF) as the matrix and was subsequently applied to the analysis of rat brain pineal gland-aCSF microdialysate. The method describes the simultaneous analysis of 23 chemically diverse compounds plus a deuterated internal standard by direct injection, requiring no dilution or extraction of the samples. The results demonstrate that this is a simple, sensitive, specific and direct approach to the qualitative analysis of these compounds in this matrix. The protocol also employs stringent MS confirmatory criteria for the detection and confirmation of the compounds examined, including exact mass measurements. The excellent limits of detection and broad scope make it a valuable research tool for examining the endogenous hallucinogen pathways in the central nervous system. We report here, for the first time, the presence of *N,N*-dimethyltryptamine in pineal gland microdialysate obtained from the rat. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: *N,N*-dimethyltryptamines; pineal gland; microdialysis; rat brain; LC/MS/MS

Introduction

In a recent review of 69 published studies reporting the detection of purported endogenous hallucinogens [*N,N*-dimethyltryptamine (DMT); 5-hydroxy-DMT (HDMT, bufotenine); 5-methoxy-DMT (MDMT)] in humans (Barker *et al.*, 2012), it was concluded that compelling mass spectral evidence exists for the confirmation of their presence in certain human biological fluids [cerebrospinal fluid (CSF; DMT and MDMT), blood (DMT and HDMT) and urine (DMT and HDMT)]. There is as yet no definitive information as to the possible normal or pathophysiological roles of DMT, HDMT or MDMT in humans or other species owing, in part, to the lack of comprehensive methods to detect and unequivocally confirm the presence of these compounds in biological tissues and fluids (Barker *et al.*, 2012). Methodology to adequately assess their synthesis and turnover, simultaneously monitoring their precursors and metabolites, is also lacking.

Original interest in endogenous hallucinogens, and DMT in particular, was motivated by the hypothesis that these compounds had a biochemical role in the heterogeneous disease state of psychosis, especially schizophrenia (for a review see Barker *et al.*, 1981a, Barker *et al.*, 2012). More recently, interest in DMT has been renewed owing to its characterization as a ligand for the sigma-1 (Fontanilla *et al.*, 2009; Su *et al.*, 2009) and trace amine receptors (Su *et al.*, 2009). Recent studies concerning the enzyme responsible for the biosynthesis of these compounds have also drawn further attention. Although the enzyme for the synthesis of the DMTs, indole-*N*-methyltransferase (INMT), was not thought to occur to any significant extent in brain (Thompson and Weinshilboum, 1998; Thompson *et al.*, 1999), studies have now shown its presence in the central nervous system, including the pineal gland (Cozzi *et al.*, 2011), motor neurons in the spinal cord (Mavlyutov *et al.*, 2012; Cozzi *et al.*, 2011) and in the retina (Cozzi *et al.*, 2011). While the enzyme is present in these tissues, there has yet to be a definitive determination of whether DMT is actually synthesized in these tissues and, if so, how it is utilized or released from the tissues under normal or altered physiological conditions.

* Correspondence to: S. A. Barker, Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, USA. E-mail: sbarker@vetmed.lsu.edu

[a] Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, 70803, USA

[b] Departments of Molecular & Integrative Physiology[1] and Neurology[2], University of Michigan Medical School, Ann Arbor, MI, 48109, USA

[c] Department of Psychiatry, University of New Mexico School of Medicine, Albuquerque, and Cottonwood Research Foundation, Gallup, New Mexico, USA

Abbreviations used: 2MTHBC, 2-methyl-1,2,3,4-THBC; CSF, cerebrospinal fluid; d_4-MDMT, $\alpha,\alpha,\beta,\beta$-tetradeutero-5-methoxy-*N,N*-dimethyltryptamine; DMT, *N,N*-dimethyltryptamine; DMTNO, DMT-*N*-oxide; HDMT, 5-hydroxy-DMT; HIAA, 5-hydroxy-IAA; HNATA, 5-hydroxy-*N*-acetyl-TA; HNMT, 5-hydroxy-*N*-methyl-TA; HTA, 5-hydroxy-TA; HTHBC, 6-hydroxy-THBC; HTRP, 5-hydroxy-tryptophan; IAA, indol-3-acetic acid; INMT, indole-*N*-methyltransferase; MAO, monoamine oxidase; MDMT, 5-methoxy-DMT; MIAA, 5-methoxy-IAA; MNMT, 5-methoxy-*N*-methyl-TA; MTA, 5-methoxy-TA; MTHBC, 6-methoxy-THBC; NMT, *N*-methyl-TA; TA, tryptamine; THBC, 1,2,3,4-tetrahydro-β-carboline; TRP, tryptophan.