Journal of Psychiatric Research 83 (2016) 220–229

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Zinc transporters protein level in postmortem brain of depressed subjects and suicide victims

Anna Rafalo-Ulinska [a, b], Joanna Piotrowska [c], Agata Kryczyk [c], Włodzimierz Opoka [c], Magdalena Sowa-Kucma [a], Paulina Misztak [a, d], Grazyna Rajkowska [e], Craig A. Stockmeier [e, f], Wojciech Datka [g], Gabriel Nowak [a, d], Bernadeta Szewczyk [a, *]

[a] Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, 31-343 Krakow, Poland
[b] Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland
[c] Department of Inorganic and Analytical Chemistry, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland
[d] Department of Pharmacobiology, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland
[e] Department of Psychiatry and Human Behavior, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, USA
[f] Department of Psychiatry, Case Western Reserve University, 10524 Euclid Avenue, Cleveland, OH 44106, USA
[g] Department of Affective Disorders, Jagiellonian University Medical College, Kopernika 21a, 31-501 Kraków, Poland

ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form
31 August 2016
Accepted 8 September 2016

Keywords:
Zinc
Zn transporters
Suicide
Major depressive disorder
Glutamate receptors
5-HT1A

ABSTRACT

Background: Major depressive disorder (MDD) is a serious psychiatric illness, associated with an increasing rate of suicide. The pathogenesis of depression may be associated with the disruption of zinc (Zn) homeostasis. In the brain, several proteins that regulate Zn homeostasis are present, including Zn transporters (ZnTs) which remove Zn from the cytosol. The present study was designed to investigate whether depression and suicide are associated with alterations in the expression of the ZnTs protein.
Methods: Protein levels of ZnT1, ZnT3, ZnT4, ZnT5 and ZnT6 were measured in postmortem brain tissue from two different cohorts. Cohort A contained 10 subjects diagnosed with MDD (7 were suicide victims) and 10 psychiatrically-normal control subjects and cohort B contained 11 non-diagnosed suicide victims and 8 sudden-death control subjects. Moreover, in cohort A we measured protein level of NMDA (GluN2A subunit), AMPA (GluA1 subunit) and 5-HT1A receptors and PSD-95. Proteins were measured in the prefrontal cortex (PFC) using Western blotting. In addition, Zn concentration was measured using a voltammetric method.
Results: There was a significant increase in protein levels of ZnT1, ZnT4, ZnT5 in the PFC in MDD, relative to control subjects, while ZnT3 protein level was decreased in MDD. There was no significant difference in the Zn concentration in the PFC between control and MDD subjects. Similarly, in the PFC of suicide victims (non-diagnosed), an increase in protein levels of ZnT1, ZnT4, ZnT5 and ZnT6 was observed. Conversely, protein levels of ZnT3 were decreased in both suicide victims and subjects with MDD, in comparison with control subjects. There was also a significant decrease in the protein level of GluA1, GluN2A, PSD-95 and 5-HT1A in MDD.
Conclusions: Our studies suggest that alterations in Zn transport proteins are associated with the pathophysiology of MDD and suicide.

1. Introduction

Major depressive disorder (MDD) is a serious mental illness with a lifetime prevalence of about 15%. One of the major causes of death in MDD is suicide. Approximately 15–25% of MDD patients attempt suicide (Mann et al., 1999; Sokero et al., 2003). Recent clinical studies report that MDD is accompanied by lower serum levels of Zn and reveal that low levels of Zn are often negatively correlated with the intensity and duration of depressive symptoms (McLoughlin and Hodge, 1990; Maes et al., 1994, 1997, 1999; Nowak and Schlegel-Zawadzka, 1999; Siwek et al., 2010). Moreover, successful antidepressant therapy led to a normalization of the serum

* Corresponding author. Department of Neurobiology, Institute of Pharmacology PAS, Smetna 12, PL 31-343 Krakow, Poland.
E-mail address: szewczyk@if-pan.krakow.pl (B. Szewczyk).

http://dx.doi.org/10.1016/j.jpsychires.2016.09.008
0022-3956/

levels of Zn, suggesting that Zn levels may be state dependent (McLoughlin and Hodge, 1990; Maes et al., 1994; Siwek et al., 2010). Furthermore, both clinical and preclinical studies showed that a dietary deficiency in Zn induces depressive symptoms (Marcellini et al., 2006; Tassabehji et al., 2008; Tamano et al., 2009; Whittle et al., 2009; Roy et al., 2010; Watanabe et al., 2010; Jacka et al., 2012; Maserejian et al., 2012; Mlyniec and Nowak, 2012; Doboszewska et al., 2015; Markiewicz-Zukowska et al., 2015). These results support the hypothesis that the pathogenesis of depression is associated with disruptions of Zn homeostasis.

There are no data so far concerning levels of serum Zn in suicide. Our previous studies performed in postmortem tissue of suicide victims showed no significant difference in total Zn levels in the prefrontal cortex (PFC) and hippocampus as compared to control subjects (Nowak et al., 2003; Sowa-Kucma et al., 2013). However, another study revealed alterations in the Zn interaction with N-methyl-D-aspartate receptors (NMDA) (Nowak et al., 2003; Sowa-Kucma et al., 2013) and a decreased in levels of GPR39-Zn(2+)-sensing receptor in the brain of suicide victims (Mlyniec et al., 2014). Thus, there is indirect evidence for a disruption in Zn homeostasis in the pathophysiology of suicide.

Zn is an essential trace element required for proper cellular function. It is a structural component of numerous proteins such as growth factors, enzymes, transcription factors and receptors, and it is important for their biological activity (Fukada et al., 2011). Hence, it is important to maintain a homeostatic intracellular concentration of Zn, since either its reduction or increase leads to the disruption of cellular functions and consequently to a variety of health problems (Pfaender and Grabrucker, 2014; Pochwat et al., 2015). Zn homeostasis is regulated by different groups of proteins including the metalotransporter family ZnT. These proteins are encoded by the solute-linked carrier (SLC) gene family: ZnT (SLC30A). Ten members of ZnT have so far been identified (Kambe et al., 2014). The ZnT family facilitates Zn efflux from the cytosol out of the cells or into intracellular compartments (Huang and Reichardt, 2001). Of 10 known ZnTs, ZnT1, 3, 4, 5 and 6 are more highly expressed in the brain. ZnT1 is localized in the plasma membrane and is responsible for the export of Zn from the cytosol to the extracellular space. ZnT3 sequesters Zn into synaptic vesicles and ZnT4 is involved in the transport of Zn to cellular compartments (Huang and Tepaamorndech, 2013). ZnT5 and ZnT6 are localized on the membrane of the Golgi apparatus as well as cytoplasmic vesicles. ZnT5 may be responsible for the transport of Zn ions across the cellular membrane and ZnT6 is involved in the trafficking of Zn during acute immune responses (Kambe et al., 2002).

The crucial role that Zn plays in the pathophysiology of depression and that most suicide attempters suffer from depression led us to design the present study to examine the levels of Zn homeostasis-regulating proteins - Zn transporters - in the pathophysiology of depression and suicide. Protein levels of ZnT1, 3, 4, 5 and 6, (transporters that are more highly expressed in the brain) were assessed in the PFC (Brodmann's area, BA10) of subjects diagnosed with MDD, in the PFC of suicide victims of unknown psychiatric history and in matched psychiatrically normal control subjects and in non-suicide control subjects. Furthermore, Zn concentration was determined in the PFC of subjects with MDD and normal control subjects.

Preclinical and clinical studies have demonstrated abnormalities in glutamatergic and serotonergic transmission in MDD (for reviews see Freudenberg et al., 2015; Kaufman et al., 2016; Ghasemi et al., 2014). On the other hand, one action of Zn is in the modulation of both glutamate (especially NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, AMPA) and serotonin (5-HT1A) receptor function (Nowak, 2015). Thus, an additional aim of the present study was to examine the expression of the NMDA receptor subunit GluN2A, AMPA receptor subunit GluA1and postsynaptic density protein-95 (PSD-95). We choose these particular proteins (GluN2A, GluA1 and PSD-95) based on reports of their important role in the function of NMDA and AMPA receptors, involvement in the pathophysiology of depression and Zn action (Feyissa et al., 2009; Freudenberg et al., 2015; Kalappa et al., 2015; Popescu, 2015; Szewczyk et al., 2015). We also measured the level of 5-HT1A receptor protein in the PFC of subjects with MDD and matched normal control subjects (cohort A). The potential correlation between the ZnTs and the glutamate receptor subunits and 5-HT1A were analyzed.

2. Materials and methods

2.1. Subjects and tissue collection

Cohort (A). Postmortem brain tissue from 10 male subjects diagnosed with MDD (mean age ± SEM, 44.8 ± 3.8) and 10 psychiatrically-normal control subjects (mean age ± SEM, 45.9 ± 3.5) (Table 1) were collected at autopsy at the Cuyahoga County Medical Examiner's Office, Cleveland, OH. The causes of death was ruled by the Medical Examiner. The Institutional Review Boards of University Hospitals Case Medical Center and the University of Mississippi Medical Center approved the protocol for recruitment, tissue collection, and interviews of the next-of-kin. Written informed consent was obtained from the legally-defined next-of-kin for collecting tissue and for informant-based diagnostic interviews regarding the deceased. Cases with a clinical history or evidence of a neurological disorder or injury were excluded. The Medical Examiner's Office examined blood and urine samples from all subjects for measurement of psychotropic medications and substances of abuse. Retrospective, informant-based psychiatric assessments were performed for all subjects as previously described (Cobb et al., 2016). A trained interviewer administered the Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID; First et al., 1995) to knowledgeable next-of-kin of all subjects to retrospectively assess the presence or absence of Axis I diagnoses according to the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (APA, 1994). Interview notes and clinical histories were reviewed independently by two licensed mental health clinicians, who assigned consensus diagnoses in conference. There is a high degree of diagnostic agreement between assessments based on interviewing next-of-kin vs. living subjects (Dejong and Overholser, 2009). Ten male control subjects did not meet criteria for a current or lifetime major mental illness. Ten male subjects were diagnosed with major depressive disorder (MDD) and were experiencing a major depressive episode in the last month of life. Four subjects had experienced one depressive episode and six had experienced 2 or more such episodes. The average age (mean ± SEM) of onset of depression was 38.0 ± 3.3 years and the average duration of illness was 4.7 ± 2.6 years (Table 2). Among the subjects with MDD, one had a prescription for an antidepressant medication in the last month of life, although no

Table 1
Demographic characteristics of control and suicide/MDD subjects (cohort A).

Parameter	Control n = 10	Major depression (MDD) n = 10	
Sex	Male	Male	–
Age (years)	45.9 ± 3.50	44.8 ± 3.77	p = 0.371
PMI (h)	22.13 ± 6.77	23.78 ± 3.05	p = 0.637
pH	6.74 ± 0.07	6.60 ± 0.0	p = 0.107

Values are mean ± S.E.M. PMI-postmortem interval; The average ages, PMI and pH values of control subjects and those with MDD were not statistically different.