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Research report

Zinc, magnesium and NMDA receptor alterations in the hippocampus of suicide victims

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ABSTRACT

Background: There is evidence for an association between suicidal behavior and depression. Accumulating data suggests that depression is related to a dysfunction of the brain's glutamatergic system, and that the N-methyl-D-aspartate (NMDA) receptor plays an important role in antidepressant activity. Zinc and magnesium, the potent antagonists of the NMDA receptor complex, are involved in the pathophysiology of depression and exhibit antidepressant activity.

Methods: The present study investigated the potency of Zn^{2+} and Mg^{2+} to [^{3}H] MK-801, which binds to the NMDA receptor channel in the hippocampus of suicide victims ($n=17$) and sudden death controls ($n=6$). Moreover, the concentrations of zinc and magnesium (by flame atomic absorption spectrometry) and levels of NMDA subunits (NR2A and NR2B) and PSD-95 protein (by Western blotting) were determined.

Results: Our results revealed that there was a statistically significant decrease (by 29% and 40%) in the potency of zinc and magnesium (respectively) to inhibit [^{3}H] MK-801 binding to NMDA receptors in the hippocampus in suicide tissue relative to the controls. These alterations were associated with increased NR2A (+68%) and decreases in both the NR2B (−46%) and PSD-95 (−35%) levels. Furthermore, lower concentrations (−9%) of magnesium (although not of zinc) were demonstrated in suicide tissue.

Conclusions: Our findings indicate that alterations in the zinc, magnesium and NMDA receptor complex in the hippocampus are potentially involved in the pathophysiology of suicide-related disorders (depression), which may lead to functional NMDA receptor hyperactivity.

1. Introduction

Depression is a serious psychiatric illness affecting approximately 17% of the population at some point in their lives and is the leading cause of disability worldwide (Duman and Voleti, 2012). Depression is associated with an increased number of suicide attempts and increased lethality. It is estimated that mood disorders, principally major depressive disorder and bipolar disorder, are associated with about 60% of all suicides (Brådvik, 2002; Brådvik and Berglund, 2005; Gibbons et al., 2005; Martinez et al., 2005). Moreover, suicide accounts for almost 2% of the world's deaths (WHO, 2000). The World Health Organization (WHO) estimates that over 120 million people worldwide suffer from depression and has, in turn, predicted that by 2020 unipolar major depression will be the second leading cause of disease or injury after ischemic heart disease (Mathews et al., 2012).

Almost 4 decades of intensive research have sought to elucidate the neurobiological basis of depression. However, a study of the biological bases of depression is still overdue. The principal aims are to increase the knowledge of the basis of the disease in order

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to better define its pathogenic process and to identify predictive biomarkers or, at least, markers able to support the diagnosis.

The data gathered during recent years suggests that abnormalities within glutamatergic transmission, especially NMDA (N-methyl-D-aspartate) receptor overactivation, are associated with more generalized mechanisms of brain dysfunction that may underlie various psychiatric disorders, including major depressive disorder. The accumulated evidence demonstrated that the functional and structural pathology of excitatory neurotransmitters have been observed in animal models of depression and clinical trials (Hansen et al., 1983; Kugaya and Sanacora 2005; Petrie et al., 2000; Sanacora et al., 2003; Zarate et al., 2002; Hashimoto, 2009; Pilc et al., 2013). Furthermore, there is evidence that NMDA receptor ligands (functional antagonists) interacting with different components of the NMDA receptor–ionophore complex produced antidepressant-like effects (Lopes et al., 1997; Panconi et al., 1993; Poleszak et al., 2007; Przegalinski et al., 1998; Trullas and Skolnick, 1990; Szewczyk et al., 2012). Furthermore, there are some clinical reports suggesting augmentation of antidepressant therapy by NMDA receptor antagonists (ketamine and CP-101,606/traxoprodil) in refractory depression (Berman et al., 2000; Zarate et al., 2006; Preskorn et al., 2008).

During the last few years, many articles have been presented indicating the important role of zinc and magnesium (inorganic NMDA receptors antagonists) in the mechanism of antidepressant therapy and/or the pathophysiology of depression (Murck, 2013; Swardfager et al., 2013). In the preclinical experiments, zinc and magnesium produced antidepressant-like activity in both tests (Decollogne et al., 1997; Kroczka et al., 2000, 2001; Poleszak et al., 2004, 2005a; Rosa et al., 2003) and some models of depression (Cieslik et al., 2007; Nowak et al., 2003a; Sowa-Kucma et al., 2008). Besides, both cations enhanced the activity of antidepressant drugs (Kroczka et al., 2001; Szewczyk et al., 2002, 2009; Cieslik et al., 2007; Poleszak et al., 2005b, 2007).

Clinical observations demonstrated a significant reduction in the serum zinc concentration in depressed patients (Hansen et al., 1983; Maes et al., 1994, 1997, 1999; Nowak et al., 1999; Siwek et al., 2010), which was normalized by successful antidepressant therapy (Maes et al., 1994; McLoughlin and Hodge, 1990; Siwek et al., 2010). Also, data concerning the blood magnesium concentration in depressed patients is inconsistent, since both increases (Frazer et al., 1983; Kirov et al., 1994; Widmer et al., 1992, 1995) and decreases (Banki et al., 1985; Hashizume and Mori, 1990; Rasmussen et al., 1989; Zięba et al., 2000; Ruljancic et al., 2013) have been observed.

Since a considerable percentage of suicide victims had suffered from depression (Bottlender et al., 2000; Hawton and van Heeringen, 2009; Pawlak et al., 2013), we hypothesized that an alteration in zinc and magnesium homeostasis might occur in brain tissue. Moreover, because hippocampal zinc and/or magnesium homeostasis in particular might be involved in the pathophysiology of affective disorders (e.g. Murck, 2002; Nowak et al., 2003c; Takeda, 2011), this brain structure was chosen for the present examination.

The main aim of the present study was to examine zinc and magnesium concentration and their potency to inhibit [^{3}H] MK-801 binding to NMDA receptors in the hippocampus of suicide victims and sudden death controls. The second aim of the study was to investigate the level of relevant subunits of the NMDA complex (NR2A, NR2B) and PSD-95 protein.

2. Experimental procedure

2.1. Tissue collection

Brain tissue from 17 suicide victims and six unexpected sudden death controls (mean age ± SEM, 35.8 ± 4.3 years for suicide and 34.3 ± 6.0 for controls) was obtained as discarded tissue at the time of autopsy by the Department of Forensic Medicine, Jagiellonian University Medical College [Grant no. 6P05B 142 20 from the State Committee for Scientific Research, approved by the Ethics Committee (2001–2004)]. According to the available medical history, both suicide and control subjects included in the study were not treated for any chronic CNS diseases (or with any psychotropic agents). The study subjects comprised seven females and 16 males (Table 1). During the autopsy, blocks (approximately 2 × 2 × 2 cm) of hippocampus were dissected, frozen and stored at −80 °C. Before analysis, each sample was divided into two parts (weight ~0.3 g). One part of the tissue was used for zinc and magnesium determination and the second for a radioligand binding assay and immunoblotting.

2.2. Radioligand binding assay

The radioligand binding assay was performed as described previously by Szewczyk et al. (2001). The hippocampal tissues were thawed in 50 volumes of ice-cold 5 mM HEPES/4.5 mM Tris buffer (pH 7.4), homogenized and then centrifuged at 20,000*g* for 20 min (0–4 °C). The resulting supernatant was discarded and the pellet was resuspended in HTS containing 1 mM EDTA before it was centrifuged at 20,000*g* for 20 min. The obtained tissue pellet was resuspended once more in 20 volumes of HTS containing 1 mM EDTA and then centrifuged at 20000*g* for 20 min. Following centrifugation, the pellet was resuspended in 5 volumes of fresh HTS and stored at −70 °C for at least 3 days before the assay. On the day of the assay, the frozen aliquots were thawed in 20 volumes of HTS and centrifuged at 20,000*g* for 20 min. Radioligand binding assays were performed in plates (MultiScreen, Millipore, Bedford, MA, USA). The incubation mixture in a final volume of 0.3 ml consisted of 240 µl membrane suspension (~0.05 mg of protein), 30 µl of a 5 nM [^{3}H] MK-801 (28.8 Ci/mmol; NEN) and a 30 µl buffer containing six concentrations (ranged 10^{-2}–10^{-7} M) of zinc or magnesium. Nonspecific binding was assessed using 100 µM phencyclidine. The assay mixture was incubated for 2 h at 25 °C and terminated by rapid vacuum

Table 1
Characteristics of suicide and sudden death control subjects.

	Age (years)	Sex (male/female)	Cause of death
1	33	M	Suicide/hanging
2	29	M	Suicide/hanging
3	21	F	Suicide/jumping
4	17	M	Suicide/hanging
5	47	M	Suicide/hanging
6	19	M	Suicide/jump under train
7	21	F	Suicide/self-poisoning/drug overdose (doxepine+clomipramine)
8	75	F	Suicide/self-mutilation
9	*17*	*M*	*Control/cranial/ brain injuries*
10	29	F	Suicide/jumping
11	20	M	Suicide/self-poisoning/drug overdose (hydroxyzine+perazine)
12	58	M	Suicide/self-poisoning/drug overdose (clomethiazole+diazepam)
13	55	M	Suicide/hanging
14	*44*	*F*	*Control/road accident*
15	20	F	Suicide/self-drowning
16	*20*	*M*	*Control/carbon monoxide poisoning*
17	55	F	Suicide/self-poisoning/drug overdose (diazepam+ethanol)
18	*54*	*M*	*Control/myocardial infarction*
19	*29*	*M*	*Control/homicide*
20	58	M	Suicide/hanging
21	*42*	*M*	*Control/myocardial infarction*
22	24	M	Suicide/jumping
23	19	M	Suicide/hanging

filtration over glass fiber filters. The filters were then washed two times with 0.1 ml of ice-cold HTS, placed in scintillation vials with 4 ml of liquid scintillation cocktail and the bound radioactivity was then measured by a Beckman LS-6500 scintillation counter.

2.3. Zinc and magnesium determination

Samples were wet-digested with nitric acid and hydrogen peroxide (microwave digestion, Milestone MLS-1200 Mega Microwave Digestion System). The determination of zinc and magnesium was carried out by a flame atomic absorption spectrometry. More specifically, a Pye Unicam SP-9 800 AA Spectrometer with a deuterium background correction (air flow – 4.5 l/min, acetylene flow – 1.1 l/min, and analytical wavelength – 213.9 nm). The relative standard deviation (RSD) of the method (the whole analytical procedure: digestion+zinc/magnesium determination) did not exceed 2.4% and the mean recovery of the zinc was 99% (SD 0.78) (Nowak et al., 2003c).

2.4. Immunoblotting

The tissue was prepared as published previously (Sowa-Kucma et al., 2008). Samples were homogenized in 2% SDS, then denaturated for 10 min at 95 °C, and centrifuged for 5 min at 10,000 rpm at 4 °C. The total protein concentration in the resulting supernatant was determined using the bicinchoninic acid method (Pierce Biotechnology, Inc., Rockford, IL, USA). The samples containing 50 μg of protein were mixed with a sample buffer (Invitrogen, Paisley, UK), fractionated by 7.5% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Invitrogen, Paisley, UK). The blots were blocked for 60 min at 4 °C with 10% bovine serum albumin (BSA) in Tris-buffered saline with 1% Tween 20 (TBS-T) and then incubated overnight at 4 °C with a primary antibody. The NR2A and NR2B subunits were labeled using rabbit polyclonal antibodies diluted 1:200 (Santa Cruz Biotechnology, Santa Cruz, CA). PSD-95 was detected on blots using a mouse monoclonal antibody (1:200 dilution, Pierce Biotechnology, Inc., Rockford, IL, USA). The membranes were then washed with TBS-T and incubated for 60 min at room temperature with a goat horseradish peroxidase-conjugated anti-rabbit IgG or anti-mouse (1:5000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA). After incubation, the blots were washed with TBS-T and developed using an enhanced chemiluminescence reaction (Western Blotting Luminol Reagent, Santa Cruz Biotechnology, Santa Cruz, CA). The NR2A, NR2B and PSD-95 signal were visualized and quantified with the FUJI-LAS 4000 system and Fuji Image Gauge v 4.0 software. As a control for the transfer and loading, the blots were incubated for 30 min with a mouse anti-β-actin antibody (1:1000 dilution, Sigma, Germany) and then processed as described above. The density of each NR2A, NR2B and PSD-95 protein band was normalized to the density of the β-actin band.

2.5. Data analysis and statistics

The data was evaluated using GraphPAD Prism software (ver. 4.0, San Diego, CA, USA). The radioligand binding data were analyzed using iterative curve fitting routines. The western blot results are presented as the NR2A or NR2B/actin ratio. All of the results are presented as means ± SEM. Group differences were assessed using the unpaired Student's *t*-test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. The effect of zinc or magnesium on [^{3}H] MK-801 binding to the NMDA complex

The radioligand receptor binding assay was used to examine the potency of Zn^{2+} and Mg^{2+} on [^{3}H] MK-801 binding to the NMDA receptor channel. [^{3}H] MK-801 is a well-characterized NMDA receptor channel antagonist and it is widely used in receptor binding studies. We used extensively washed neuronal membrane preparations from the human hippocampus. The present data demonstrated a significant increase in the IC_{50} value of zinc [by 29 ± 7%; from 0.343 ± 0.016 mM to 0.441 ± 0.025 mM; $t(21)=2.244$, and $P=0.0358$] and magnesium [by 40 ± 10%; from 0.977 mM ± 0.094 mM to 1.371 ± 0.101 mM; $t(21)=2.181$, and $P=0.0407$] inhibition of [^{3}H] MK-801 binding to NMDA receptors between the control and suicide tissue (Fig. 1, Table 2). There were no alterations in specific [^{3}H] MK-801 binding in both brain regions between the control and suicide tissue (Table 2).

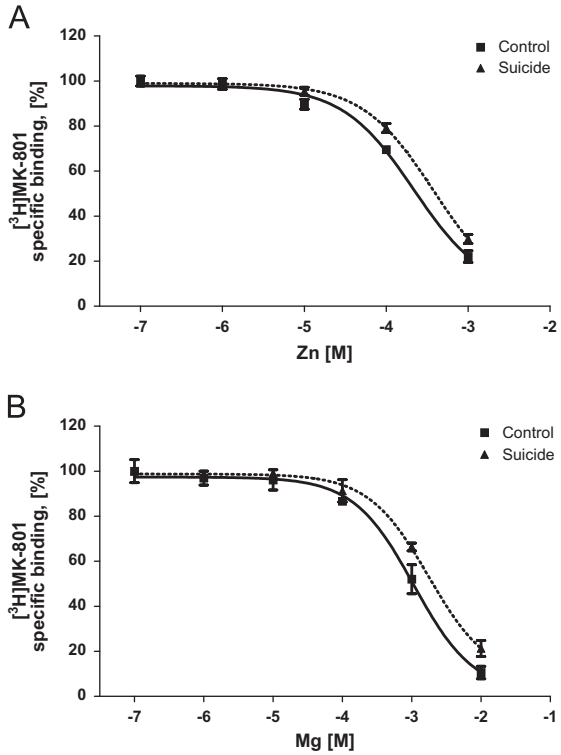

Fig. 1. Representative curves of the effect of zinc (A) and magnesium (B) on [^{3}H] MK-801 binding to the NMDA receptors in human hippocampal membranes of suicide victims and sudden death controls.

Table 2
The potency of zinc (A) and magnesium (B) to inhibit [^{3}H] MK-801 binding to the NMDA receptor in the hippocampus of suicide victims and sudden death controls. Data are expressed as IC_{50} (mM) values and represent mean ± SEM of 6 (control) or 17 (suicide) subjects.

	IC_{50} [mM]	%	Specific binding (pmol/g tissue)	*n*
ZINC				
Control	0.343 ± 0.016	100	3.554 ± 0.299	6
Suicide	**0.441 ± 0.025**[a]	**129**	3.703 ± 0.226	17
MAGNESIUM				
Control	0.977 ± 0.094	100	3.719 ± 0.368	6
Suicide	**1.371 ± 0.101**[a]	**140**	3.640 ± 0.225	17

[a] $p < 0.05$ vs. control.

Table 3
Concentration of zinc and magnesium in the hippocampus of suicide victims and sudden death controls. Data are expressed in micrograms Mg^{2+}/g tissue and represent mean ± SEM of 6 (control) or 17 (suicide) subjects.

	ZINC	%	MAGNESIUM	%
Control	10.67 ± 0.30	100	126.9 ± 3.0	100
Suicide	10.93 ± 0.30	102	**115.7 ± 2.7**[a]	**91**

[a] $p < 0.05$ vs. control.

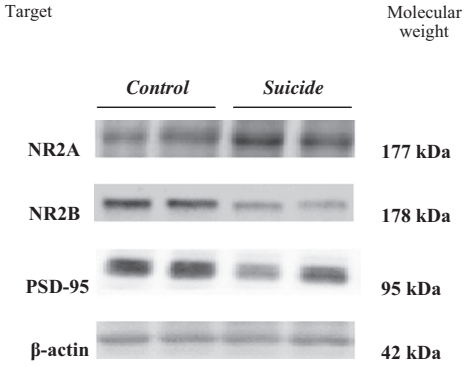

Fig. 2. Immunoblots of NR2A, NR2B, PSD-95 and β-actin from representative subjects used in the analysis. Each well was loaded with 50 μg total of protein.

3.2. Zinc and magnesium determination

Measurement of ion concentrations by flame atomic absorption spectrometry revealed a significant (by 9 ± 2.1%) decrease of magnesium in the hippocampus of suicide victims when compared to the controls [$t(21) = 2.277$ and $P = 0.0334$, (Table 3)]. On the other hand, there were no changes in zinc concentration [$t(21) = 0.4813$ and $P = 0.6353$, (Table 3)].

3.3. Immunoblotting of NR2A, NR2B subunits of the NMDA complex and PSD-95 protein

Immunoreactive bands corresponding to molecular masses of 177, 178, 95 and 42 kDa were revealed for NR2A, NR2B, PSD-95 and β-actin, respectively (Fig. 2). As shown in Fig. 3, the amount of NR2A immunoreactivity from suicide victims was significantly higher (68 ± 18.8% increase) than that of the control subjects in the hippocampus [$t(21) = 2.580$ and $P = 0.0189$, (Fig. 3A)]. Conversely, there was a robust reduction in the level of NR2B protein in suicide victims when compared to the controls [46 ± 6% decrease, $t(21) = 2.281$ and $P = 0.0331$, (Fig. 3B)]. Similarly, the protein level of PSD-95 was lowered in suicide victims [36 ± 10.8% decrease, $t(21) = 2.137$, and $P = 0.0445$, (Fig. 3C)].

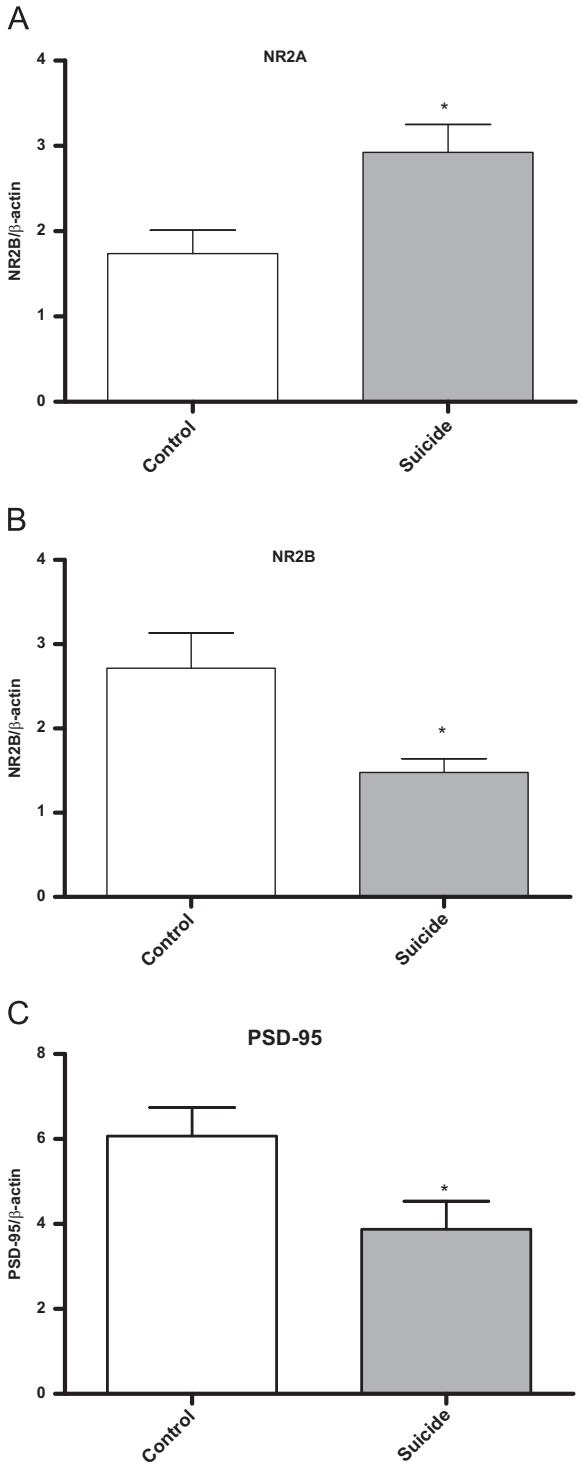

Fig. 3. Amounts of NR2A (A), NR2B (B) and PSD-95 (C) immunoreactivities in the hippocampus of suicide victims ($n = 17$) and sudden death controls ($n = 6$). Results (mean ± SEM) are shown as NR2A, NR2B or PSD-95/β-actin ratio. *$p < 0.05$ vs. control (Student's t-test).

4. Discussion

In recent years, there has been an increasing interest in the involvement of zinc and magnesium in the pathophysiology of depression. Moreover, both of these ions appear to have therapeutic potential in clinical depression. It was found that depressed patients showed a significantly lower serum zinc level than psychiatrically normal controls (Maes et al., 1994, 1997; Nowak et al., 1999; McLoughlin and Hodge, 1990; Siwek et al., 2010). The beneficial effect of zinc supplementation to antidepressant therapy has been demonstrated [(Nowak et al., 2003b; Ranjbar et al., in press; Siwek et al., 2009) see (Lai et al., 2012; Swardfager et al., 2013) for review]. Furthermore, experimental zinc deficiency induced depression-like behavior in animals (Młyniec et al., 2012; Młyniec and Nowak, 2012; Whittle et al., 2009; Tassabehji et al., 2008; Tamano et al., 2009). Zinc demonstrated antidepressant-like activity in a number of preclinical tests and models: the forced swim test (Kroczka et al., 2000, 2001; Rosa et al., 2003; Szewczyk et al., 2002), an olfactory bulbectomy (Nowak et al., 2003a), plus chronic mild stress (Sowa-Kucma et al., 2008) or chronic unpredictable stress (Cieslik et al., 2007).

Additionally, zinc enhanced the antidepressant-like activity of antidepressants in preclinical paradigms (Kroczka et al., 2001; Szewczyk et al., 2002, 2009; Cieslik et al., 2007; Cunha et al., 2008). Likewise, joint administration of zinc and NMDA receptor antagonists enhanced their antidepressant efficacy (Szewczyk et al., 2010).

An increasing number of studies have also indicated a significant relationship between magnesium and depression. Magnesium deficiency is believed to contribute to mood disorders in spite of the fact that blood tests of depressed patients provide frequently inconsistent results. The serum/plasma levels of magnesium ions in depressives may be elevated, decreased or remain unchanged (e.g. Camardese et al., 2012; Eby and Eby, 2006, 2010; Imada et al., 2002). It was recently reported that decreased intracellular Mg^{2+} has been observed within the anterior cingulate cortex in serotonin selective reuptake inhibitor treatment-resistant depressed patients (Iosifescu et al., 2008). In humans, a cross-sectional study found an inverse association between a dietary intake of magnesium and depression symptoms (Jacka et al., 2009; Yary et al., 2013). Additionally, the mood-improving efficacy of magnesium supplementation was observed in patients with major depression and postpartum depression (Eby and Eby, 2006). A deficiency of magnesium has been reported to increase depression-like behavior in mice (Muroyama et al., 2009; Singewald et al., 2004; Spasov et al., 2008), which is correlated with a decrease in the brain magnesium concentration (Whittle et al., 2011). Moreover, magnesium possesses potent antidepressant-like properties in the forced swim test in rodents (Decollogne et al., 1997; Poleszak et al., 2004, 2005a). In addition, the anti-immobility action of some antidepressants as well as NMDA receptor antagonists is enhanced by joint administration with magnesium (Decollogne et al., 1997; Poleszak, 2007; Poleszak et al., 2005b, 2007). The interrelationship between magnesium and NMDA receptor antagonists (ketamine) in depression was thoroughly discussed by Murck (2013).

The present study shows a reduction in the potency (due to an increase in the IC_{50} value) of zinc and magnesium in interacting with the NMDA receptor in the hippocampal tissue of suicide victims when compared to sudden death controls. These findings are consistent with our previous study (Nowak et al., 2003c), which revealed that the same changes in the interaction between zinc and NMDA might be involved in the psychopathology underlying suicidality. Furthermore, the hippocampal concentration of magnesium was reduced in suicides with no alterations in the zinc level. Thus, the present and previous data indicate the reduced ability of zinc and magnesium to inhibit the NMDA receptor function in the hippocampus of suicide victims, and if we consider also the reduced magnesium level, the hypersensitivity of the NMDA receptor in the brain of a suicide victim might appear.

All this data confirms the importance of zinc and magnesium in mood disorders, but a question then arises regarding there being a link between changes in the affinity of these ions and the arrangement of NMDA receptor subunits. It is known that the NMDA receptor complex functions as a heterotetramer of two glycine-binding NR1 subunits and two glutamate-binding NR2 subunits. Alternative splicing of three exons in NR1 subunits and differential expression of NR2 subunits from four separate genes (A–D) results in substantial molecular diversity of NMDA receptors during development, both across brain regions and during physiologic and pathologic conditions (Hatton and Paoletti, 2005). The subunit composition of NMDA receptors determines their biophysical and pharmacological properties, including sensitivity to MK-801, Zn^{2+} and Mg^{2+} (Paoletti and Neyton, 2007). It is now established that MK-801 binds to a site accessible only when the ion channel is open and preferentially binds with the same affinity to NR2A or NR2B-containing receptor complexes (Paoletti and Neyton, 2007). It is also known that the Zn^{2+} ion at lower concentrations (IC_{50} 20–30 nM) binds to the NR2A subunit in a high-affinity voltage-independent manner. Likewise, zinc binds to the NR2B subunit but with a >100-fold lower affinity (IC_{50} 20–100 μM) and does not bind to NR2C or NR2D subunits. The other inorganic NMDA receptor modulator, magnesium, is also more potent at inhibiting NR1/NR2A and NR1/NR2B receptors than NR1/NR2C and NR1/NR2D receptors. The affinity of Mg^{2+} to NR2A and NR2B is relatively lower ($IC_{50}\sim20$ μM) than Zn^{2+} (Paoletti and Neyton, 2007). Based on the above data, it could be suggested that the changes observed and noted in our study of the potency of zinc and magnesium to inhibit [^{3}H] MK-801 binding can be a consequence of an alteration in the composition of NMDA subunits. Indeed, some studies have indicated that depression or depression-like behavior might be related to alterations in NMDA receptor subunit concentrations (Feyissa et al., 2009; Karolewicz et al., 2009; Tokita et al., 2012). Because of this, we decided to investigate the NR2A and NR2B protein concentration, as well as the protein, PSD-95, which is responsible for the anchoring and scaffolding of the NMDA to postsynaptic density in the tissue of our subjects.

Our study demonstrates that the amount of NR2A is significantly elevated in the hippocampus of suicide subjects when compared to sudden death controls. On the other hand, the NR2B and PSD-95 protein levels were decreased. Thus, the reduced potency of zinc or magnesium to inhibit the NMDA receptor in the hippocampus was accompanied by an increase of NR2A and a reduction in NR2B subunits of this receptor complex. This pattern of changes in the NR2A vs. NR2B subunit levels may also explain the lack of differences between suicide and the controls of the specific [^{3}H] MK-801 binding to the NMDA channel in the hippocampus shown in the present studies. Likewise, binding studies using a variety of radioligands showed changes only in glutamate sites with no alterations in the ion channel or glycine sites of the NMDA complex in brain samples from suicides (Holemans et al., 1993; Nowak et al., 1995a, 1995b; Palmer et al., 1994; Dean et al., 2001; Meador-Woodruff et al., 2001).

As discussed above, zinc inhibits at low nanomolar concentrations of NR2A and with higher concentrations (micromolar) the NR2B subunit of the NMDA receptor complex (see Sensi et al., 2011, for review). Keeping in mind that the NMDA receptor complex possessing mostly NR2A subunits are predominant in the synapse and NR2B are located extrasynaptically, it can be speculated that the synaptic NMDA receptors are elevated while the extrasynaptic are reduced in the hippocampus of suicide victims (e.g. see Vizi et al., 2013).

Since enhancement of neurogenesis and a decrease in neurodegeneration-related processes are mediated by synaptic (NR2A) NMDA receptors and the opposite mechanism is mediated by extrasynaptic (NR2B) NMDA receptors, these changes detected in NMDA receptor subunits in the hippocampus may underlay the complex pathophysiology of suicidality or/and depression as well as the efficacy of applied therapies. However, further detailed studies are needed to examine this issue.

The altered function of the NMDA receptor in the pathophysiology of experimental depression was also indicated in our previous studies which showed the increased potency of glycine to interact with the NMDA receptor in the cortex of rats subjected to chronic mild stress (Nowak et al., 1998) or the forced swim stress (Nowak et al., 1995b) and diminution of this potency in other models, chronic severe stress (Nowak et al., 1998) or olfactory bulbectomy (Nowak, 1996). On the other hand, chronic treatment with antidepressants (and zinc) reduced the potency of glycine to interact with the NMDA receptor complex in the rodent cortex (Cichy et al., 2009; Nowak et al., 1993; Paul et al., 1993, 1994). Thus, according to our first introductory hypothesis, antidepressant therapy reduced the activity of NMDA glutamate

receptors, while the pathophysiology (depression and animal models) might be due to an enhancement of this signaling (Skolnick et al., 1996). The current results are in agreement with this line of thinking.

In spite of the limitations of the study concerning the subjects' description (the lack of psychiatric diagnosis, limited medical history), these data for the first time indicate alterations in the zinc, magnesium and NMDA receptor complex in the hippocampus of suicide victims, and, as depression is the major cause of suicide, possibly in the hippocampus of depressed subjects.

Role of funding source

The funding source had no role in study design, in the collection, analysis and interpretation of data, in writing of the report and in the decision to submit the paper for publication.

Conflict of interest

All authors declare that there are no actual or potential conflicts of interest that could inappropriately influence this work.

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