EXTRACELLULAR MATRIX ALTERATIONS IN THE KETAMINE MODEL OF SCHIZOPHRENIA

GABRIELA MATUSZKO, [a] SEBASTIANO CURRELI, [a,b] RAHUL KAUSHIK, [a] AXEL BECKER [c] AND ALEXANDER DITYATEV [a,d,e]*

[a] *Molecular Neuroplasticity, German Center for Neurodegenerative Diseases (DZNE), 39120 Magdeburg, Germany*

[b] *Neuroscience and Brain Technologies Department, Istituto Italiano di Tecnologia, Genova 16163, Italy*

[c] *Institute of Pharmacology and Toxicology, Faculty of Medicine, Otto-von-Guericke-University Magdeburg, 39120 Magdeburg, Germany*

[d] *Center for Behavioral Brain Sciences (CBBS), 39120 Magdeburg, Germany*

[e] *Medical Faculty, Otto-von-Guericke University, 39120 Magdeburg, Germany*

Abstract—The neural extracellular matrix (ECM) plays an important role in regulation of perisomatic GABAergic inhibition and synaptic plasticity in the hippocampus and cortex. Decreased labeling of perineuronal nets, a form of ECM predominantly associated with parvalbumin-expressing interneurons in the brain, has been observed in post-mortem studies of schizophrenia patients, specifically, in brain areas such as prefrontal cortex, entorhinal cortex, and amygdala. Moreover, glial ECM in the form of dandelion clock-like structures was reported to be altered in schizophrenia patients. Here, we verified whether similar abnormalities in neural ECM can be reproduced in a rat model of schizophrenia, in which animals received subchronic administration of ketamine to reproduce the aspects of disease related to disrupted signaling through N-methyl-D-aspartate receptors. Our study focused on two schizophrenia-related brain areas, namely the medial prefrontal cortex (mPFC) and hippocampus. Semi-quantitative immunohistochemistry was performed to evaluate investigate ECM expression using *Wisteria floribunda* agglutinin (WFA) and CS56 antibody, both labeling distinct chondroitin sulfate epitopes enriched in perineuronal nets and glial ECM, respectively. Our analysis revealed that ketamine-treated rats exhibit reduced number of WFA-labeled perineuronal nets, and a decreased intensity of parvalbumin fluorescence in mPFC interneurons somata. Moreover, we found an increased expression of CS56 immunoreactive form of ECM. Importantly, the loss of perineuronal nets was revealed in the mPFC, and was not detected in the hippocampus, suggesting regional specificity of ECM alterations. These data open an avenue for further investigations of functional importance of ECM abnormalities in schizophrenia as well as for search of treatments for their compensation. © 2017 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: prefrontal cortex, hippocampus, perineuronal net, CS56, WFA, schizophrenia.

INTRODUCTION

Neural extracellular matrix (ECM) is a well-organized complex molecular structure surrounding neurons and glia cells. Perineuronal net (PNN) is a mesh-like form of ECM, ensheathing somata and proximal dendrites of specific subclasses of neurons. Hyaluronic acid constitutes the backbone of PNN, which binds to chondroitin sulfate proteoglycans (Dityatev et al., 2010a). This complex is stabilized by glycoprotein tenascin-R and link proteins (Yamaguchi, 2000). Tenascin-R carries the human natural killer 1 (HNK-1) carbohydrate, which plays a pivotal role in regulation of perisynaptic inhibition (Saghatelyan et al., 2000). A lack of tenascin-R or HNK-1 leads to reduction in perisomatic inhibition and impaired long-term potentiation (Saghatelyan et al., 2000, 2001, 2003). LTP in tenascin-R knockout mice might be rescued by agonists of GABA_A receptors, an antagonist of postsynaptic GABA_B receptors and HNK-1 carbohydrate mimetics (Bukalo et al., 2007). In addition to regulation of perisomatic GABAergic inhibition and LTP, PNNs appeared to control excitability of fast-spiking interneurons (Dityatev et al., 2007).

These findings on the role of ECM in regulation of GABAergic innervation and activity are particularly relevant in the context of schizophrenia, because impaired GABAergic inhibition is one of the putative causes of this disease (Beasley et al., 2002; Reynolds et al., 2004; Sakai et al., 2008). Reduced levels of 67-kDa glutamate decarboxylase (GAD67) mRNA in parvalbumin (PV)-expressing interneurons are frequently reported in the PFC of schizophrenic patients (Benes et al., 1996; Volk et al., 2001; Volk and Lewis, 2002). Importantly, human post-mortem brain studies revealed the reduction of PNN density in multiple brain areas, including prefrontal and entorhinal cortices, and amygdala (Pantazopoulos et al., 2010). Also, dandelion clock-like structures, presumably a glial form of ECM, were

*Correspondence to: Alexander Dityatev, German Center for Neurodegenerative Diseases (DZNE), Leipziger Str. 44, Haus 64, 39120 Magdeburg, Germany. Fax: +49-391-6724530.

E-mail address: Alexander.Dityatev@dzne.de (A. Dityatev).

Abbreviations: ECM, extracellular matrix; mPFC, medial prefrontal cortex; NGS, normal goat serum; PBS, phosphate buffer solution; PNN, perineuronal net; PV, parvalbumin; ROIs, regions of interest; WFA, *Wisteria floribunda* agglutinin.

markedly reduced in the amygdala of schizophrenia patients (Pantazopoulos et al., 2015). Genetic studies also revealed several interesting connections between ECM encoding genes and schizophrenia. Common variation of neurocan, one of the chondroitin sulfate proteoglycans, has been reported to be associated with this mental disorder (Mühleisen et al., 2012) and confirmed by a more recent study (Ripke et al., 2013). Another genetic study on schizophrenia patients has reported single nucleotide polymorphism of β-1,3-glucuronyltransferase 2 (B3GAT2), an enzyme involved in HNK-1 biosynthesis, as a schizophrenia risk allele, associated with decreased cortex volume in patients (Kahler et al., 2011). Also matrix metalloprotease MMP-16, link protein HAPLN4 and neuroglycan C were identified as genes associated with schizophrenia (Ripke et al., 2013; Consortium SWGotPG, 2014). Furthermore, 50% reduction of mRNA and protein levels of the ECM glycoprotein Reelin was found in several brain areas, including PFC and hippocampus, in schizophrenia patients (Costa, 1998; Fatemi et al., 1999; Guidotti et al., 2000).

However, one should be cautious with interpretation of the mentioned post-mortem brain studies, as ECM alterations in patient brains may at least partially reflect increased sensitivity to proteolysis during postmortem period or be related to drug treatment of patients. Thus, it is important to verify human findings using animal models of schizophrenia. Moreover, models would allow one to search for pharmacological treatments to restore ECM and to dissect the impact of ECM on positive, negative and cognitive symptoms in schizophrenia. Our study aimed to investigate ECM alterations, in particular in the number and prominence of PNNs and CS56$^+$ glial ECM, using a pharmacological rat model based on sub-chronic administration of NMDA receptor antagonist ketamine (Becker et al., 2003). Ketamine-treated rats have impaired latent inhibition and reduced percentage of non-aggressive interactions in sociability test (Becker et al., 2003), and are considered as a model of schizophrenia predominantly reflecting negative symptoms of the disease (Chindo et al., 2012). Our immunohistochemical analysis revealed a reduced number of PNNs and increased intensity of CS56$^+$ ECM in the prefrontal cortex of ketamine-treated rats.

EXPERIMENTAL PROCEDURES

Animals

Sprague–Dawley (MolTac:SD, Taconic Denmark, SPD) male rats were used in all experiments. The animals were kept in controlled laboratory conditions: at 20 ± 2 °C, air humidity 55–60% and 12-h day/night cycle (lights on at 6 a.m.). The rats were housed in group of five animals in Macrolon IV cages, with free access to food pellet (Altronim 1326) and tap water.

Ketamine treatment

Ketamine treatment was done according to the protocol of Becker et al. (2009). Eight-week-old animals were subjected to sub-chronic administration of ketamine (ketamine hydrochloride, Astrapin, Pfaffen-Schwabenhein, Germany). Ketamine (30 mg/kg b.w.) or 0.9% NaCl vehicle were injected in two sessions of 5 days, with daily injections separated by 2-day break between sessions. Injections were made intraperitonally (IP) at a volume of 1 ml/100 g of animal weight.

Social interaction test

Social interaction test was performed as described previously (Becker et al., 2003, 2009). Briefly, two days after the final injection, control rats and ketamine-treated rats were housed singly in Macrolon II cages with food and water *ad libitum* for 12 days. Two weeks after the last ketamine injection animals were familiarized to the open-field arena (100 × 100 × 40 cm) in two trials of 7 min each two days prior to social interaction test. The day prior to testing, rats were allocated to test partners on the basis of pretreatment and body weight. The difference between the two partners was within 20 g. During the test, animal pairs were placed in arena for 7 min. Total time of social interaction was measured and scored as non-aggressive and aggressive interactions between two animals. Data are represented as the time span of non-aggressive behavior as a percentage of total social interaction time.

Immunohistochemistry

Two days after social interaction test, animals were deeply anesthetized (400 mg/kg b.w. chloral hydrate injection) and perfused transcardially with 4% PFA. Brains were incubated in 4% PFA containing phosphate buffer solution (PBS), cryoprotected in 30% sucrose PB solution for 48 h, frozen in 100% 2-methylbutan at −80 °C and sliced in 50-μm-thick coronal sections. Floating sections were kept in solution (1 part ethylenglycol, 1 part of glycerin, 2 parts of PBS pH = 7.2). For each staining condition, three sections per brain area of each animal were selected. All sections were washed in 120 mM phosphate buffer pH = 7.2 (PB).

For double PV and WFA staining, medial prefrontal cortex (mPFC) and dorsal hippocampal sections were permeabilized with PB containing 0.5% Triton X-100 (Sigma T9284) for 10 min at RT, followed by application of blocking solution (PB supplemented with 0.1% Triton X-100 and 5% normal goat serum (NGS) (Gibco 16210-064) for 1 h at RT. Afterward, sections were incubated for 48 h in the presence of primary reagents: biotinylated *Wisteria floribunda* agglutinin (WFA) and rabbit anti-parvalbumin antibody (staining conditions are described in Table 1). Sections were washed three times in PB followed by incubation with secondary reagents, streptavidin Alexa 488 conjugate and goat anti-rabbit Alexa 546-conjugated secondary antibodies, overnight at 4 °C. Sections were washed in PB and stained with Hoechst 3342 (1 mg/ml in DMSO, 1:500, Sigma B2261) and mounted on SuperFrost glasses with Fluoromount (Sigma F4680).

For triple PV, WFA and HNK-1 staining, mPFC sections were permeabilized with PB buffer containing