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A lack of GluN2A-containing NMDA receptors confers a vulnerability to redox dysregulation: Consequences on parvalbumin interneurons, and their perineuronal nets

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ABSTRACT

The GluN2A subunit of NMDA receptors (NMDARs) plays a critical role during postnatal brain development as its expression increases while Glun2B expression decreases. Mutations and polymorphisms in GRIN2A gene, coding for GluN2A, are linked to developmental brain disorders such as mental retardation, epilepsy, schizophrenia. Published data suggest that GluN2A is involved in maturation and phenotypic maintenance of parvalbumin interneurons (PVIs), and these interneurons suffer from a deficient glutamatergic neurotransmission via GluN2A-containing NMDARs in schizophrenia.

In the present study, we find that although PVIs and their associated perineuronal nets (PNNs) appear normal in anterior cingulate cortex of late adolescent/young adult GRIN2A KO mice, a lack of GluN2A delays PNN maturation. GRIN2A KO mice display a susceptibility to redox dysregulation as sub-threshold oxidative stress and subtle alterations in antioxidant systems are observed in their prefrontal cortex. Consequently, an oxidative insult applied during early postnatal development increases oxidative stress, decreases the number of parvalbumin-immunoreactive cells, and weakens the PNNs in KO but not WT mice. These effects are long-lasting, but preventable by the antioxidant, *N*-acetylcysteine. The persisting oxidative stress, deficit in PVIs and PNNs, and reduced local high-frequency neuronal synchrony in anterior cingulate of late adolescent/young adult KO mice, which have been challenged by an early-life oxidative insult, is accompanied with microglia activation.

Altogether, these indicate that a lack of GluN2A-containing NMDARs alters the fine control of redox status, leading to a delayed maturation of PNNs, and conferring vulnerability for long-term oxidative stress, microglial activation, and PVI network dysfunction.

1. Introduction

Parvalbumin-expressing interneurons (PVIs) are fast-spiking GABAergic neurons that form inhibitory synapses onto either cell body or axon initial segment of pyramidal neurons. Via feed-forward and feed-back inhibitions, these interneurons exert a precise temporal control on the information flowing through their target neurons. Interconnected PVIs constitute a neuronal network able to synchronize the excitatory state of large numbers of neurons (Bartos et al., 2007). Thus, PVIs coordinate the activity of neuronal assemblies in high-frequency synchrony (Cardin et al., 2009; Fuchs et al., 2007; Massi et al., 2012; Sohal et al., 2009), thereby modulating the processing of information required for sensory perception, working memory, attention, learning and memory, and social behavior (Billingslea et al., 2014; Hu et al., 2014).

Anomalies in PVIs and their associated networks are key pathological features of schizophrenia (SZ) (Beasley and Reynolds, 1997; Lewis et al., 2012). Moreover, the specialized extracellular matrix (perineuronal nets, PNNs), which envelops many PVIs, is also abnormal in this disease (Enwright et al., 2016; Mauney et al., 2013; Pantazopoulos et al., 2015). The immature developmental gene expression profile of PVIs in SZ (Gandal et al., 2012) suggests a defect in the proper phenotypic maturation of these interneurons, which are particularly sensitive to perturbations during brain development (Steullet et al., 2016). Some of the proposed mechanisms underlying PVI anomalies in SZ are aberrant wiring of PVIs within neuronal networks, impaired glutamatergic inputs onto these interneurons and deleterious effects of neuroinflammation and oxidative stress (Lewis et al., 2012; Steullet et al., 2016). Impaired NMDA receptor (NMDAR)-dependent signaling, which represents a major pathological pathway in SZ (Coyle et al., 2012; Poels et al., 2014; Steiner et al., 2013), affects PVIs (Behrens et al., 2007) and neuronal network activity (Carlen et al.,

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2012; Homayoun and Moghaddam, 2007; Kocsis et al., 2013; Korotkova et al., 2010). These interneurons are particularly sensitive to NMDAR antagonists during their development and maturation (Abekawa et al., 2007; Powell et al., 2012; Wang et al., 2008). However, the contribution of the different subtypes of NMDARs to the effect of general NMDAR antagonists is not well established.

NMDARs, which are ionotropic glutamate receptors, are heterotetramers consisting of two obligatory GluN1 subunits and two variable subunits (GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, or GluN3B). The subunit composition of NMDARs determines the kinetic of calcium entrance upon receptor activation and the nature of the activated intracellular signaling (Paoletti et al., 2013). Therefore, distinct subtypes of NMDARs convey different functions. The GluN2A subunit, whose expression increases during early postnatal life, is involved in cortical network refinement during development via its actions on structural and functional synaptic plasticity (Cho et al., 2009; Fagiolini et al., 2003). Cortical PVIs undergo an early postnatal and activity-dependent switch of the GluN2 subunit composition of NMDARs, with GluN2A becoming more numerous than GluN2B subunits (Matta et al., 2013; Zhang and Sun, 2011). The postnatal increase of GluN2A in PVIs coincides with the maturation of these interneurons and the PNN formation. Studies using antagonists targeting preferentially either GluN2A - or GluN2B-containing NMDARs suggest that GluN2A, not GluN2B, contribute to PVI maturation and phenotypic maintenance (Kinney et al., 2006; Zhang and Sun, 2011). A disrupted function of GluN2A-containing NMDARs, as suggested by genetic studies, might therefore result in aberrant maturation and function of PVIs in some patients. Indeed, a few mutations in GRIN2A, the gene encoding GluN2A, have been identified in SZ (Hardingham and Do, 2016; Tarabeux et al., 2011). The locus containing GRIN2A is also associated with schizophrenia in a large genome wide association study (Consortium, 2014). The high-risk polymorphisms of GT repeats on the GRIN2A promoter give rise to reduced transcription (Liu et al., 2015), and reduced GluN2A expression, have been reported in prefrontal cortex (Beneyto and Meador-Woodruff, 2008) and PVIs (Bitanihirwe et al., 2009).

Although pharmacological manipulations support a role of GluN2A in development and phenotyping maintenance of PVIs, a demonstration of such function via genetic deletion of GRIN2A was missing. Moreover, how the GluN2A-mediated signaling disruption impact PVIs remain unclear. Redox dysregulation/oxidative stress, which is widely reported in schizophrenia (Do et al., 2009; Flatow et al., 2013; Kim et al., 2016; Yao and Keshavan, 2011), is a common endpoint of many genetic and environmental risk factors leading to defects in PVIs and their associated PNNs in the prefrontal cortex, namely the anterior cingulate cortex (ACC) (Steullet et al., 2017). In a recent perspective article, we briefly disclosed that PVI and PNN deficits are also accompanied with oxidative stress in GRIN2A KO mice. Here, we further explored the relationship between PVIs, PNNs and oxidative stress in the ACC of mice lacking GluN2A-containing NMDARs during postnatal development.

2. Methods

2.1. Animals

GRIN2A KO mice (Sakimura et al., 1995) were provided by A. Lüthi (University of Lausanne). They were backcrossed with C57BL/6J mice through many generations and housed under a 12-h light-dark cycle in groups of 3–5 individuals/cage. Experiments were performed on both sexes and approved by the Local Veterinary Office.

2.2. GBR12909 and N-acetylcysteine treatments

GBR12909 (GBR, 10 mg/kg i.p.) (BioTrend, Switzerland) was injected daily from postnatal days 10 to 20. The vehicle solution, a phosphate buffer saline solution (PBS), was used in control animals. *N*-acetylcysteine (NAC) (PharmaNAC, BioAdvantexPharma, Canada) was provided in drinking water (2.4 g/L) to the lactating mothers from postnatal days 7 to 20 (das Neves Duarte et al., 2012).

2.3. Perfusion and tissue preparation

For immunohistochemistry, mice were anesthetized and perfused with 4% paraformaldehyde. Coronal frozen sections (50-μm thick) of fixed brains were prepared and stored in ethylene glycol at − 20 °C until use. For mRNA quantification, Western blots and GSH measurements, brains were quickly dissected out and sliced (2-mm thick) using a brain matrix. Slices were immediately stored at − 80 °C until use. Tissue from the medial prefrontal cortex was extracted from frozen brain slices using a tissue punch.

2.4. Immunohistochemistry

Triple immunolabeling for oxidative stress, parvalbumin (PV), and perineuronal nets (PNNs) was performed as followed. An antibody against 8-oxo-2′-deoxyguanosine (8-oxo-dG), a product of DNA oxidation, was used as oxidative marker, while the PNNs were labeled with the lectin *Wisteria floribunda* Agglutinin (WFA), which binds the *N*-acetylgalactosamines in chondroitin sulfate proteoglycans (Berretta et al., 2015; Wang and Fawcett, 2012). Brain sections containing the anterior cingulate cortex (ACC) were first incubated with PBS + Triton 0.3% + sodium azide (1 g/L) containing 2–3% normal horse serum, then placed for 48 h in a solution with a mouse monoclonal anti-8-oxo-dG (1:400; AMS Biotechnology, Switzerland) and a rabbit polyclonal anti-parvalbumin (PV) (1:2500; Swant Inc., Switzerland) primary antibody together with the biotin-conjugated WFA (1:2000; Sigma, Switzerland). Sections were then washed, incubated with fluorescent secondary antibody conjugates: goat anti-mouse Alexa 488 (1:300; Life Technologies, USA), goat anti-rabbit CY3 (1:300; Chemicon International, USA) and streptavidin 405 conjugate (1:300; Millipore Corporation, USA). For dual immunolabeling of Iba1 and CD68 to label microglia, normal donkey serum (2%) was used as blocking agent. Primary antibodies were a goat polyclonal anti-Iba1 (1:1000; Abcam, UK) and a rat monoclonal anti-CD68 (1:1500; Abcam) antibody. Secondary antibody conjugates were a chicken anti-goat Alexa 594 (1:300; Life Technologies, USA) and a chicken anti-rat Alexa 488 (1:300; Life Technologies, USA).

Sections were visualized and processed with a Zeiss confocal microscope equipped with × 10, × 40 and × 63 Plan-NEOFLUAR objectives. All peripherals were controlled with LSM 710 Quasar software (Carl Zeiss AG, Switzerland). With the triple immunolabeling of 8-oxo-dG, PV, and WFA, Z-stacks of 12 images (with a 2.13 μm interval) were scanned (1024 × 1024 pixels). Images were filtered with a Gaussian filter to remove background noise and sharpen cell profile contour. Only the inner 8 images of Z-stacks were used for the analyses. The stack images were merged in a tif file with Imaris, and further analyzed with Image J. Analyses were performed in a delineated region of interest (ROI) comprising the ACC (cg1) by an observer unaware of the experimental groups. For PV-IR, the number of PV-IR cell bodies was counted and the mean intensity of labeling (arbitrary unit) was measured separately within PV-IR cell bodies and PV-IR processes. PV-IR processes included all PV-labeled voxels that were not part of the identified PV-IR cell bodies. The mean intensity (arbitrary unit) was used as a measure for 8-oxo-dG-IR and WFA labeling. Because the image acquisition, the defined ROI, and the image analysis were slightly different to those used in Steullet et al. (2017), the intensity of 8-oxo-dG (arbitrary unit) and the numbers of PV-IR cells/ROI in the present study were not identical to those reported in the above article. However irrespective of the analytical method used, the differences between experimental groups were similar. The number of PV-IR neurons surrounded by WFA-labeled PNNs was also counted in IMARIS software