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Subchronic pharmacological and chronic genetic NMDA receptor hypofunction differentially regulate the Akt signaling pathway and Arc expression in juvenile and adult mice

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ABSTRACT

NMDA receptor (NMDAR) hypofunction is a compelling hypothesis for the pathophysiology of schizophrenia, because in part, NMDAR antagonists cause symptoms in healthy adult subjects that resemble schizophrenia. Therefore, NMDAR antagonists have been used as a method to induce NMDAR hypofunction in animals as a pharmacological model of schizophrenia. Serine racemase-null mutant (SR−/−) mice display constitutive NMDAR hypofunction due to the lack of D-serine. SR−/− mice have deficits in tropomyosin-related kinase receptor (TrkB)/Akt signaling and activity regulated cytoskeletal protein (Arc) expression, which mirror what is observed in schizophrenia. Thus, we analyzed these signaling pathways in MK801 sub-chronically (0.15 mg/kg; 5 days) treated adult wild-type mice. We found that in contrast to SR−/− mice, the activated states of downstream signaling molecules, but not TrkB, increased in MK801 treated mice. Furthermore, there is an age-dependent change in the behavioral reaction of people to NMDAR antagonists. We therefore administered the same dosing regimen of MK801 to juvenile mice and compared them to juvenile SR−/− mice. Our findings demonstrate that pharmacological NMDAR antagonism has different effects on TrkB/Akt signaling than genetically-induced NMDAR hypofunction. Given the phenotypic disparity between the MK801 model and schizophrenia, our results suggest that SR−/− mice more accurately reflect NMDAR hypofunction in schizophrenia.

1. Introduction

N-methyl-D-aspartate receptor (NMDAR) hypofunction is a compelling hypothesis for the pathophysiology of schizophrenia (Coyle et al., 2010). This hypothesis has been developed partially by the observation that NMDAR antagonists, such as phencyclidine and ketamine, reproduce all of the schizophrenia symptom domains in healthy human subjects including hallucinations, negative symptoms and cognitive symptoms (Itil et al., 1967; Javitt, 2009). Therefore, NMDAR antagonists have been used as a tool to induce NMDAR hypofunction in experimental animals as a pharmacologic model of schizophrenia (Adell et al., 2012; Eyjolfsson et al., 2006; Javitt and Zukin, 1991; Wiescholleck and Manahan-Vaughan, 2013). These pharmacological models exhibit hyperlocomotion, stereotypies (Hoffman, 1992) and social withdrawal (Zou et al., 2008), as well as long-term potentiation (LTP) and learning deficits (Manahan-Vaughan et al., 2008).

There is also genetic and biochemical evidence to support NMDAR hypofunction as a key etiological component of schizophrenia. Recent large-scale, exome sequencing (Fromer et al., 2014) and genome-wide association (GWAS) studies (Timms et al., 2013; Ripke and for the Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) have identified de novo mutations and genetic loci, respectively, in genes encoding proteins involved in glutamatergic transmission, including NMDAR subunits, with increased risk for schizophrenia. A single nucleotide polymorphism (SNP) in the enzyme serine racemase (SR), which produces D-serine, the forebrain NMDAR co-agonist, was among the risk alleles significantly associated with schizophrenia (Morita et al., 2007; Ripke and for the Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Furthermore, SR and D-serine are reduced in schizophrenia (Bendikov et al., 2007; Hashimoto et al., 2003; Nishikawa, 2011). Thus, our laboratory generated serine racemase-null mutant (SR−/−) mice that display constitutive NMDAR hypofunction due to the lack of D-serine (Basu et al., 2009).

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Similar to schizophrenia, SR −/− mice have reduced cortico-hippocampal volume and ventricular enlargement that is accompanied by decreased dendritic spine density and complexity in these regions (Balu et al., 2013; Puhl et al., 2014). Further investigation revealed that SR −/− mice have impaired neurotrophic signaling that parallels what is observed in schizophrenia, including brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB)/Akt/glycogen synthase 3 kinase (GS3K) cascade (Balu et al., 2013). In addition, we found that activity-regulated cytoskeleton-associated protein (Arc), which is genetically associated with schizophrenia (Kirov et al., 2012; Ripke and for the Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), is reduced in the hippocampus of adult SR −/− mice (Balu and Coyle, 2014). Because BDNF expression, Akt signaling and Arc levels are regulated by NMDAR activity, we therefore analyzed this pathway and Arc in a pharmacological NMDAR hypofunction model.

Among NMDAR antagonists, (+)-MK801 hydrogen maleate (MK801) has a favorable profile because it has an extremely high (10–100 fold higher than PCP and ketamine) affinity to (Kornhuber and Weller, 1997), and a high selectivity for the PCP binding site of the NMDAR (Wong et al., 1986) whereas PCP also binds to the dopamine D2 receptor (Seeman et al., 2005). Furthermore, there is a notable age-dependent change in the behavioral response of people to NMDAR antagonists. In children, PCP and ketamine do not produce psychosis, which are typical for these drugs in adult (Spear, 2000). This age dependency of NMDAR antagonists' effects is also interesting because schizophrenia typically has its symptomatic onset in early adulthood. Although there are some studies that analyzed the age-dependent difference of NMDAR antagonists on rodent behavior (Boulay et al., in press; Sircar and Soliman, 2003), there are few that examined intracellular signaling.

Thus, we analyzed TrkB/Akt/GS3K signaling pathways and Arc in MK801 sub-chronically (0.15 mg/kg; o.d; 5 days) treated adult wild-type mice and SR −/− mice to elucidate these two model difference on the TrkB signaling, Akt signaling and Arc. We administered the same dosing regimen of MK801 to juvenile mice (3–4 weeks old) and compared them to juvenile SR −/− mice.

2. Materials and methods

2.1. Animals

Wild-type (WT) and constitutive SR −/− mice were generated as previously described (Basu et al., 2009). The serine racemase null mutation of the first coding exon has been backcrossed for over 10 generations onto a C57BL/6J background. SR +/− parents were bred to produce WT and SR −/− offspring. Male mice were used for all experiments as they exhibit a much more robust phenotype than females. 3–7 month old mice were utilized for adult mice, and 3–4 week old mice were for juvenile mice. The animals were housed in a temperature- (22 °C) and humidity-controlled facility with a 12/12 h light/dark cycle and provided with food and water ad libitum. All animal procedures were approved by the McLean Hospital Institutional Animal Care and Use Committee.

2.2. Drug treatment

WT mice were administered either vehicle (saline) or MK801 via intraperitoneal (i.p.) injection at a volume of 10 ml/kg body weight once daily for 5 days at 10 to 11 A.M. MK801 was obtained from Sigma-Aldrich (M107, St. Louis, MO, USA). MK801 was dissolved in sterile isotonic saline at a dosage of 0.15 mg/kg.

2.3. Western blot analysis

Immunoblotting was performed as modification of the previously described method (Balu and Coyle, 2011). MK801-treated animals were sacrificed 90 min after the last injection, and their brains were quickly removed. Both lobes of the hippocampus were collected. The tissue was flash frozen and stored at −80 °C until homogenizing. Brain tissue was briefly homogenized by sonication (>10 s) in extraction buffer (60-mM Tris buffer, 2% sodium dodecyl sulfate, 0.1% phosphatase inhibitor, pH 6.8). Protein content was determined by a colorimetric assay based upon the Bradford method using Bio-Rad dye reagent (Bio-Rad Life Sciences, Hercules, CA). Prior to gel loading, samples were heated to 95 °C for 5 min. Samples were electrophoretically separated on an SDS-12.5% polyacrylamide gel. Nitrocellulose membranes (Bio-Rad, Hercules, CA) were blocked with 5% nonfat dry milk (Shaw's; Boise, ID) in 0.05% Tween-20/Tris-buffered saline and then incubated with primary antibody overnight at 4 °C. The primary antibodies (Cell Signaling Technologies; Danvers, MA) raised in rabbit were used at the following dilutions: p-AKT1 (ser) (1:1000), AKT1 (1:1000), p-GS3K (1:1000), GS3K (1:1000), p-TrkB (1:2000), and β-actin (1:8000; Abcam; Cambridge, MA). Arc (Santa Cruz Biotechnology; Santa Cruz, CA) and TrkB (Millipore; Temecula, CA) were detected using a mouse monoclonal primary antibody (1:200, 1:2000, respectively). After incubation with goat anti-rabbit (1:5000; Abcam, Cambridge, MA) or rabbit anti-mouse (1:1500; Abcam, Cambridge, MA) horseradish peroxidase-conjugated secondary antibodies, immunocomplexes were visualized by chemiluminescence using Western Lightning-ECL (Perkin Elmer; Waltham, MA). Semi-quantitative assessment of protein bands was executed by computerized densitometry using Image Labo Software (Bio-Rad, Hercules, CA). Chemiluminescent values of the protein of interest were divided by its corresponding β-actin chemiluminescent values.

2.4. Statistical analyses

The results were compared using unpaired Student t-test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Constitutive genetic NMDAR hypofunction and sub-chronic pharmacologic NMDA receptor antagonism have opposite effects on TrkB/Akt/GS3K signaling in adult mice

We first measured the phosphorylation status of the TrkB receptor, which is upstream of the Akt/GS3K pathway, as a way to determine the activation state of the receptor. In agreement with our previous findings, adult SR −/− mice showed a 20% reduction of phosphorylated TrkB (pTrkB; Fig. 1A; $p < 0.001$), without a change in the total amount of TrkB. In contrast, MK801-treated mice did not show a significant change in the phosphorylation state of the TrkB receptor compared to vehicle treated mice (Fig. 1B; $p = 0.4$).

The amounts of phosphorylated-Akt1 (p-Akt) and phosphorylated-GS3K (p-GS3K), which are the active states of the enzymes, were reduced in the hippocampus of adult SR −/− mice compared to WT mice (Fig. 1C, E; pAkt1, $p < 0.05$; pGS3Kα: $p < 0.05$; pGS3Kβ, $p < 0.01$), in line with our previous results. There was no difference in the total amounts of Akt or GS3K (Fig. 1C, E). However, in adult MK801 treated mice, the phosphorylation states of these proteins increased compared to saline treated mice (Fig. 1D, F; p-Akt1, $p < 0.05$; p-GS3Kα, $p < 0.05$; p-GS3Kβ, $p < 0.05$), with no difference in the total amounts of Akt and GS3K (Fig. 1D,F).

3.2. Juvenile SR −/− mice showed reduced TrkB activation and Akt/GS3K signaling

Because we were interested in whether the reductions in TrkB and Akt/GS3K signaling were age-dependent, we examined the phosphorylation states of these proteins in juvenile SR −/− mice. Similar to adult SR −/− mice, juvenile SR −/− animals exhibited deficits of equal magnitude in pTrkB (Fig. 2A; $p < 0.05$), pAkt1