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Juvenile treatment with mGluR2/3 agonist prevents schizophrenia-like phenotypes in adult by acting through GSK3β

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ABSTRACT

Prodromal memory deficits represent an important marker for the development of schizophrenia (SZ), in which glutamatergic hypofunction occurs in the prefrontal cortex (PFC). The mGluR2/3 agonist LY379268 (LY37) attenuates excitatory N-methyl-D-aspartate receptor (NMDAR)-induced neurotoxicity, a central pathological characteristic of glutamatergic hypofunction. We therefore hypothesized that early treatment with LY37 would rescue cognitive deficits and confer benefits for SZ-like behaviors in adults. To test this, we assessed whether early intervention with LY37 would improve learning outcomes in the Morris Water Maze for rats prenatally exposed to methylazoxymethanol acetate (MAM), a neurodevelopmental SZ model. We found that a medium dose of LY37 prevents learning deficits in MAM rats. These effects were mediated through postsynaptic mGluR2/3 via improving GluN2B-NMDAR function by inhibiting glycogen synthase kinase-3β (GSK3β). Furthermore, dendritic spine loss and learning and memory deficits observed in adult MAM rats were restored by juvenile LY37 treatment, which did not change prefrontal neuronal excitability and glutamatergic synaptic transmission in adult normal rats. Our results provide a mechanism for mGluR2/3 agonists against NMDAR hypofunction, which may prove to be beneficial in the prophylactic treatment of SZ.

1. Introduction

Schizophrenia (SZ) is a debilitating psychiatric disorder that affects ~ 1% of the population (Marin, 2016). Current pharmacological treatments for SZ are often limited in efficacy, especially for cognitive symptoms. Although the neural basis of SZ is poorly understood, emerging evidence suggests that disturbances in glutamate synaptic signaling in the prefrontal cortex (PFC) stand out as a strong possible mechanism underlying cognitive impairments (Snyder and Gao, 2013). Specifically, in early brain development, pathological genetic variation combined with environmental risk factors trigger NMDAR hypofunctioning, which may eventually compromise cortical function and delay the onset of cardinal symptoms until adulthood (Murray et al., 2014). This neurodevelopmental model proposes that cognitive impairments are central to the pathophysiology of SZ, resulting from aberrant brain maturational processes (Cannon, 2015; Murray et al., 2004).

Indeed, SZ-like phenotypes are generated in neurodevelopmental animal models, providing useful tools to assess the alterations of normal brain developmental trajectories. For example, prenatal exposure to methylazoxymethanol acetate (MAM) reproduces some of the core features of SZ, including NMDAR dysfunction, GABAergic deficits, as well as cognitive impairments (Du and Grace, 2013; Moore et al., 2006; Snyder et al., 2013). Importantly, NMDAR hypofunction and cognitive impairments in MAM model begin during peri-pubertal stage (Gulchina et al., 2017; Le Pen et al., 2006; Snyder and Gao, 2013). It is conceivable that this early alteration represents a critical window for therapeutic interventions considering the importance of NMDARs in the refinement of excitatory synapses during postnatal development (Marin, 2016; Monaco et al., 2015); and if this period has been missed, interventions targeting NMDAR hypofunction in SZ would likely be compromised. However, targeting NMDARs in the early stage of development has potentially risky side effects due to the neurotoxic effects of glutamate (Herndon and Coyle, 1977; Liu et al., 1996).

Group II metabotropic glutamate receptors (mGluR2/3) have received significant interest as potential non-dopaminergic SZ drug targets (Delevich et al., 2015; Muguruza et al., 2016; Xing et al., 2016). Specifically, activation of mGluR2/3 negatively modulates

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synaptic transmission by reducing presynaptic glutamate release (Mateo and Porter, 2007). In contrast, postsynaptic mGluR2/3 enhances neuronal excitability (Jin et al., 2018) and AMPARs and NMDARs function directly (Li et al., 2015a, 2015b, 2017; Tyszkiewicz et al., 2004; Wang et al., 2013; Xi et al., 2011). Although clinical trials of mGluR2/3 agonists in adult patients with SZ have yielded controversial results (Downing et al., 2014; Patil et al., 2007), emerging data suggest that pharmacological activation of mGluR2/3 modulates glutamate neurotransmission and affects NMDAR function, holding the promise of this treatment strategy for the glutamate hypofunction hypothesis (Li et al., 2015a). Intriguingly, previous studies have revealed that mGluR2/3 levels undergo marked fluctuations in the developing brain (Catania et al., 1994; Defagot et al., 2002), which may have ramifications for the action of mGluR2/3 agonists during the early postnatal critical period (Ross et al., 2000).

In our recent study (Li et al., 2017), we found that targeting mGluR2/3 in the early stage can correct NMDAR hypofunction and has a beneficial effect for cognition deficits observed in adult MAM rats. However, the mechanism of how it works was untested. Using the MAM model, here we test the hypothesis that early interventions with mGluR2/3 agonist prevent SZ-like electrophysiological, morphological, and cognitive deficits in adults via restoring NMDAR signaling during a critical postnatal period.

2. Materials and methods

2.1. Animals

Adult pregnant Sprague–Dawley rats were purchased from the Charles River Laboratories International (Wilmington, MA) and were intraperitoneally (i.p.) injected with 25 mg/kg MAM or saline control (SAL) on gestational day 17 (GD 17) as described previously (Goto and Grace, 2006; Lodge et al., 2009; Snyder et al., 2013). Pups were weaned on a postnatal day 21 (P21) and rehoused 2–4 per cage under a light/dark cycle of 12 h, with food and water *ad libitum*. For all experiments, rats aged P12–21 as juveniles, P28–45 as adolescents based on others' (Spear, 2000) and our previous studies (Snyder et al., 2013; Wang and Gao, 2009, 2010; Xing et al., 2016). All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Drexel University College of Medicine Animal Care and Use Committee.

2.2. Drugs and treatment

LY379268 (LY37, Cat. No. 2453), Ro 25-6981 (Cat. No. 1594/1), and tetrodotoxin (TTX) (Cat. No. 1078/1) were obtained from Tocris Bioscience. DNQX (6,7-dinitroquinoxaline-2,3-dione, Cat. No. 2379-57-9), Picrotoxin (Cat. No. 124-87-8) and all other chemicals were purchased from Sigma-Aldrich.

For LY37 treatment, the drug was dissolved in saline (10 mg/ml) as the stock solution and stored at −20 °C. The stock solution was freshly diluted to 2 mg/ml with saline on the days of injection. Final concentration for each rat was 0.1, 0.3 or 1.0 mg/kg. SAL- or MAM rats were treated with either control saline or LY37 intraperitoneally every other day from P21 to P27 at 10 a.m.

2.3. Morris water maze

Spatial learning and memory were assessed by the Morris water maze (MWM) as described before (Snyder et al., 2013). Briefly, male SAL and MAM rats were i.p. injected with different doses of LY37 (0.1, 0.3 and 1.0 mg/kg, once daily for five consecutive days) or saline vehicle control 1 h before each MWM training day beginning at age P17. The different groups of rats were required to locate a fixed hidden platform using visual cues, and training consisted of four trials each day for five consecutive days before the probe memory test. Each rat was allowed to swim until it found the submerged platform in a 1-m diameter water maze or until 60 s elapsed. The average of the four trials was used as the mean latency to reach the platform on each training day. The probe memory test was performed 24 h after the last training session and was used to evaluate the animals' ability to recall the learned platform location. During probe testing, the platform was removed from the pool, and the rats were allowed to swim for 60 s while being tracked by a computerized video system. The amount of time spent swimming in the previous platform location quadrant minus the time spent swimming along the pool edge was calculated and compared among different groups.

2.4. Tissue collection and protein preparation

After deeply anesthetized with Euthasol (Virbac Animal Health, Fort Worth, Texas), rats were sacrificed by decapitation, and the brains were quickly removed. The forebrain tissue containing medial PFC (mPFC) was dissected and the whole-cell lysates were processed as before (Li et al., 2015b; Xi et al., 2011; Xing et al., 2016). Briefly, tissue was homogenized in ice-cold lysis buffer [in mM: 20 Tris-HCl, pH 7.4, 150 NaCl, 1 EDTA, 1 sodium orthovanadate, 0.1 phenylmethylsulfonyl fluoride, 50 sodium fluoride, 10 sodium pyrophosphate, 20 glycerophosphate], with 0.1% sodium dodecyl sulfate (m/V), 0.01% Triton X (V/V), 0.25% sodium deoxycholate (m/V), 1 μg/ml leupeptin, 1 μg/ml aprotinin and 1 μg/ml pepstatin]. After centrifugation at 13′000 g for 15 min at 4 °C, the supernatant was transferred into new tubes and stored in −80 °C for future use. For some experiments, synaptic membrane protein was prepared as described before (Li et al., 2015b, 2017; Snyder et al., 2013). After perfused with ice-cold buffer (in mM: 320 sucrose, 4 HEPES-NaOH buffer, pH 7.4, 2 EGTA, 1 sodium orthovanadate, 0.1 phenylmethylsulfonyl fluoride, 50 sodium fluoride, 10 sodium pyrophosphate, 20 glycerophosphate, with 1 mg/ml leupeptin and 1 mg/ml aprotinin), the mPFC tissue was dissected and homogenized in sucrose buffer and then centrifuged at 1000 g for 10 min to remove large cell fragments and nuclear materials. The resulting supernatant was centrifuged at 17′000 g for 15 min to obtain cytoplasmic proteins. The pellet was resuspended in homogenization buffer and centrifuged at 17′000 g for 15 min to produce synaptosomes. The synaptosomal fraction then was hypoosmotically lysed and centrifuged at 25′000 g for 20 min to yield synaptosomal plasma membranes. Homogenization buffer was added to the pellet to make the final samples, which were then stored at −80 °C for future use.

2.5. Western blotting

A bicinchoninic acid (BCA) protein assay was performed to determine protein concentration. The protein sample was mixed with laemmli sample buffer, boiled for 5 min, and separated on a 7.5% SDS-PAGE gel. After electrophoresis, proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Billerica, MA, USA). The membranes were blocked for 1 h with 5% fat milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) and incubated overnight with primary antibodies at 4 °C. Blots were probed with anti-mouse NR1 (Thermo Fisher Scientific Cat# 32-0500, RRID: AB_2533060, 1:5000), anti-rabbit NR2A (Millipore Cat# 04-901, RRID: AB_1163481, 1:4000), anti-mouse NR2B (Millipore Cat# 05-920, RRID: AB_417391, 1:2000), anti-rabbit mGluR2 (Millipore Cat# 07-261, RRID: AB_2116167, 1:2000), anti-rabbit mGluR3 (Abcam, Cat# ab140741, RRID: AB_2716689, 1:4000), anti-mouse