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Research Paper

LY395756, an mGluR2 agonist and mGluR3 antagonist, enhances NMDA receptor expression and function in the normal adult rat prefrontal cortex, but fails to improve working memory and reverse MK801-induced working memory impairment

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ABSTRACT

Targeting group II metabotropic glutamate receptors (mGluR2/3) has been proposed to correct the dysfunctional glutamatergic system, particularly NMDA receptor (NMDAR) hypofunction, for treatment of schizophrenia. However, how activation of mGluR2/3 affects NMDAR function in adult animals remains elusive. Here we show the effects of LY395756 (LY39), a compound acting as both an mGluR2 agonist and mGluR3 antagonist, on the NMDAR expression and function of normal adult rat prefrontal cortex (PFC) as well as working memory function in the MK801 model of schizophrenia. We found that in vivo administration of LY39 significantly increased the total protein levels of NMDAR subunits and NR2B phosphorylationin the PFC, along with the amplitude of NMDAR-mediated miniature excitatory postsynaptic currents (mEPSC) in the prefrontal cortical neurons. Moreover, LY39 also significantly increased mTOR and pmTOR expression, but not ERK1/2, Akt, and GSK3β, suggesting an activation of mTOR signaling. Indeed, the mTOR inhibitor rapamycin, and actinomycin-D, a transcription inhibitor, blocked the enhanced effects of LY39 on NMDAR-mEPSCs. These results indicate that LY39 regulates NMDAR expression and function through unidentified mTOR-mediated protein synthesis in the normal adult rat PFC. However, this change is insufficient to affect working memory function in normal animals, nor to reverse the MK801-induced working memory deficit. Our data provide the first evidence of an in vivo effect of a novel compound that acts as both an mGluR2 agonist and mGluR3 antagonist on synaptic NMDAR expression and function in the adult rat PFC, although its effect -on PFC-dependent cognitive function remains to be explored.

1. Introduction

Cognitive dysfunction in schizophrenia (SCZ) usually persists into asymptomatic periods and is not reversed by dopaminergic antipsychotic treatments (Kahn and Sommer, 2015; Millan et al., 2012; Weinberger and Gallhofer, 1997). Although the mechanism associated with cognitive impairment of SCZ remains unclear, the N-methyl-D-aspartate receptor (NMDAR) hypofunction hypothesis is widely linked with cognitive deficits (Lisman et al., 2008; Snyder and Gao, 2013). Unfortunately, current antipsychotic drugs almost exclusively target dopaminergic and serotoninergic systems without direct effects on glutamatergic receptors (Howes and Kapur, 2009; Miyamoto et al., 2005; Seeman, 2011). Therefore, the searching for novel treatments that can recover the dysfunctional glutamate system is a promising strategy in drug development for treatment of SCZ, especially for those patients whose symptoms are unrelated to dopaminergic dysfunction (Howes and Kapur, 2014).

Abbreviations: 3,5-DHPG, 3,5-dihydroxyphenylglycine; AC, adenylate cyclase; ACPD, 1-amino-1,3-dicarboxycyclopentane; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPH, amphetamine; APDC, dihydroxyphenylglycine; cAMP, cyclic-adenosine monophosphate; DCG-IV, (2S,1′R,2′ R,3′R)-2-(2,3-dicarboxycyclopropyl) glycine; ERK1/2, extracellular signal-regulated kinase1/2; GABA, gama-aminobutyric acid; GSK3β, glycogen synthase kinase 3β; I.P., intraperitoneal; mGluRs, metabotropic glutamate receptors; NMDAR, N-methyl-D-aspartate receptor; PKA, protein kinase A; PKC, protein kinase C; SNAP, soluble NSF activating protein; SNARE, soluble NSF activating protein receptor; SNP, single nucleotide polymorphism; SCZ, schizophrenia; VAMP, vesicle-associated membrane protein.

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We are particularly interested in the group II metabotropic glutamate receptors (mGluRs) because mGluR2/3 agonists have exhibited therapeutic potential for different types of neurological and psychiatric disorders, particularly SCZ, due to their regulatory effects on the glutamatergic system (Conn et al., 2009; Fell et al., 2012; Li et al., 2015; Mezler et al., 2010; Moghaddam and Adams, 1998).

Activation of mGluR2/3 can directly decrease presynaptic glutamate release via $G_{\alpha i/o}$-mediated inhibition of the adenylate cyclase–cyclic AMP-protein kinase A pathway (Cartmell and Schoepp, 2000; Moghaddam, 2004; Schoepp et al., 1999). However, recent studies have also provided evidence that mGluR2/3 agonists enhance postsynaptic excitatory receptor function, including both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and NMDARs. In particular, we found that selective mGluR2/3 agonist LY379268 reverses dizocilpine (MK-801)-induced postsynaptic NMDA dysfunction via activation of the glycogen synthase kinase 3β (GSK3β) pathway (Xi et al., 2011). In addition, LY379268 also increases surface expression and function of AMPARs through activation of extracellular signal-regulated kinase1/2 (ERK1/2) and GSK3β signaling pathways in cultured prefrontal cortical neurons (Wang et al., 2013).

LY395756 (LY39) is a novel compound that serves as both an mGluR2 agonist and mGluR3 antagonist ($K_i = 0.165$ and 0.302 μM, respectively) (Ceolin et al., 2011; Lucas et al., 2013). We recently observed that LY39 also exhibited a strong effect on AMPAR expression in cultured neurons (Wang et al., 2013). Given that mGluR2 and mGluR3 display distinct regional, cell-type, and synaptic specificities (Petralia et al., 1996; Tamaru et al., 2001; Wright et al., 2013), we speculate that mGluR2 and mGluR3 receptors may play different regulatory roles in synaptic transmission, and consequently have differential clinical implications. Indeed, evidence suggests that mGluR2 mainly mediates antipsychotic activity in SCZ (Fell et al., 2008; Woolley et al., 2008), while mGluR3 exerts neuroprotective effects (Durand et al., 2013).These findings prompt us to further examine whether targeting mGluR2 with LY39 would have an effect on NMDAR function in adult rat PFC and whether this action is sufficient to affect cognitive function. Our results showed that LY39 significantly increased NMDAR expression in the PFC and function of the prefrontal cortical neurons, and this enhancement was mediated by activation of mammalian target of rapamycin (mTOR)-dependent protein synthesis. However, this effect is insufficient to reverse MK801-induced working memory deficits.

2. Materials and methods

2.1. Animals and treatments

Adult male Sprague Dawley rats (P90, 330–360 g) were purchased from Charles River Laboratories (Wilmington, MA). They were housed under conditions of constant temperature (21–23 °C) and humidity on a reverse 12 h light/dark cycle with food and water available ad libitum. Animals were allowed to adapt to the new environment for 2 days before the experiments. The animal procedures were performed in accordance with the National Institutes of Health (NIH) animal use guidelines and were approved by the Institutional Animal Care and Use Committee of Drexel University College of Medicine.

LY39, (+)-MK801 maleate, and actinomycin-Dwere purchased from Tocris Bioscience (Minneapolis, MN) and Rapamycin was purchased from LC Laboratories (Woburn, MA).

2.2. Acute treatment for Western blot assay

LY39 at a dose of 0.3, 1.0, or 3.0 mg/kg (single dose, intraperitoneally, i.p.) was administered 1 h before the rats were sacrificed for tissue collection as a peak brain concentration of a similar compound LY379268 could be reached within 30 min post-I.P. administration (Bond et al., 2000). For electrophysiological recording, a single injection of LY39 (3.0 mg/kg, I.P.) was administered 1 h before the animals were sacrificed for ex vivo slices. In both cases, saline solution (0.9% sodium chloride) was used as a vehicle control with 5 animals in each treatment group. In additional experiments, rapamycin (1 mg/kg) (Autry et al., 2011), actinomycin-D (act-D, 0.5 mg/kg) (Miller et al., 2014), or saline was administered 30 min before LY39 or saline injection. All animals were deeply anesthetized with Euthasol (0.2 ml/kg, Virbac Animal Health) and were decapitated per the approved IACUC protocol.

2.3. Adult rat perfusion

To preserve brain tissue quality, adult rats were anesthetized with Euthasol and then rapidly perfused with intracardiac injection of 60 ml ice-cold sucrose buffer (in mM: 320 sucrose, 4 HEPES–NaOH buffer, pH 7.4, 2 EGTA, 1 sodium orthovanadate, 0.1 phenylmethylsulfonyl fluoride, 10 sodium fluoride, 10 sodium pyrophosphate) for Western blotting, or of 60 ml cold artificial cerebrospinal fluid (ACSF, in mM: 124 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 $CaCl_2$, 1 $MgSO_4$, 26 $NaHCO_3$, and 10 dextrose, pH 7.4) for electrophysiology.

2.4. Synaptic membrane protein collection

The animals were decapitated, and the brains were quickly removed. The forebrain containing the prelimbic area was dissected, homogenized in cold lysis buffer (in mM: 320 sucrose, 4 HEPES–NaOH buffer, pH 7.4, 2 EGTA, 1 sodium orthovanadate, 0.1 phenylmethylsulfonyl fluoride, 10 sodium fluoride, 10 sodium pyrophosphate, with 1 μg/ml leupeptin and 1 μg/ml aprotinin). The tissues were centrifuged at 1000 *g* for 10 min at 4 °C to remove large cell fragments and nuclear materials, and the resulting supernatant was centrifuged again at 15,000 *g* for 15 min at 4 °C to harvest cytoplasmic proteins in the supernatant. The pellet from this spin was resuspended in lysis buffer and centrifuged at 15,000 *g* for an additional 15 min at 4 °C to produce synaptosomes. The synaptosomal fraction was then hypoosmotically lysed and centrifuged at 25,000 *g* for 30 min at 4 °C to collect the crude synaptosomal pellet. Lysis buffer was added to the pellet to make the final samples, which were then stored in −80 °C for future use, or aliquots were made and stored at −20 °C for immediate use.

2.5. Western blots

A bicinchoninic acid (BCA) protein assay was performed to determine protein concentration. The protein sample was mixed with 4× laemmli and lysis buffer, boiled for 5 min, and separated on a 7.5% SDS-PAGE gel. After electrophoresis, proteins were transferred to Immobilon PVDF membranes (Millipore, IPVH00010). The membrane was blocked in 5% nonfat milk and probed with primary antibodies at 4 °C overnight. Each blot was used to probe multiple antibodies, including anti-mouse NR1 (Invitrogen, 32–0500, 1:5000), anti-rabbit NR2A (Millipore, 04–901, 1:2500), anti-mouse NR2B (Millipore, 05–920, 1:2000), anti-rabbit pNR2B-Tyr1472 (CALBIOCHEM, 1:1000), anti-rabbit pNR2B-Ser1303 (Millipore, 07–398, 1:1000), anti-rabbit Akt (Cell Signaling Technology, 2938S, 1:1000), anti-rabbit pAkt-Ser473 (Cell Signaling Technology, 4060S, 1:1000), anti-rabbit ERK1/2 (Cell Signaling Technology, 4695S, 1:10,000), anti-rabbit pERK1/2 (Cell Signaling Technology, 4370S, 1:10,000), anti-rabbit GSK3b (Cell Signaling Technology, 9315S, 1:20,000), anti-rabbit pGSK3b-Ser9 (Cell Signaling Technology, 9336S, 1:2000), anti-rabbit mTOR (Cell Signaling Technology, 2983S, 1:2000), anti-rabbit pmTOR-S2448 (Cell Signaling Technology, 2983S, 1:1000), anti-4E-BP1 (Cell Signaling Technology, 9452, 1:1000), anti-p4E-BP1 (Cell Signaling Technology, 236B4, 1:500–1000), and anti-mouse actin (Sigma, A5316, 1:100,000) served as a loading control. Membranes were stripped for 30 min with Restore Western blot Stripping Buffer (Thermo Scientific, 21063) between each different set of primary antibodies. Specifically, we probed phospho-antibodies first and then probed antibodies against each corresponding total protein. The blots were incubated with horseradish peroxidase-coupled anti-rabbit or