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Disruption of Akt signaling decreases dopamine sensitivity in modulation of inhibitory synaptic transmission in rat prefrontal cortex

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ABSTRACT

Akt is a serine/threonine kinase, which is dramatically reduced in the prefrontal cortex (PFC) of patients with schizophrenia, and a deficiency in Akt1 results in PFC function abnormalities. Although the importance of Akt in dopamine (DA) transmission is well established, how impaired Akt signaling affects the DA modulation of synaptic transmission in the PFC has not been characterized. Here we show that Akt inhibitors significantly decreased receptor sensitivity to DA by shifting DA modulation of $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) in prefrontal cortical neurons. Akt inhibition caused a significant decrease in synaptic dopamine D2 receptor (D2R) levels with high-dose DA exposure. In addition, Akt inhibition failed to affect DA modulation of IPSCs after blockade of β-arrestin 2. β-arrestin 2-mediated interaction of clathrin with D2R was enhanced by co-application of a Akt inhibitor and DA. Taken together, the reduced response in DA modulation of inhibitory transmission mainly involved β-arrestin 2-dependent D2R desensitization.

1. Introduction

Akt, also known as protein kinase B (PKB), is a serine/threonine kinase that plays an important role in the pathogenesis of schizophrenia (SZ) (Bajestan et al., 2006; Emamian et al., 2004; Schwab et al., 2005; Xu et al., 2007). Akt1 protein levels were significantly reduced in brain tissues from patients with SZ, particularly in the prefrontal cortex (PFC) (Emamian, 2012; Emamian et al., 2004; Thiselton et al., 2008; Zhao et al., 2006). The PFC is known to be important in working memory and other cognitive functions, and PFC dysfunction is responsible for many neuropsychiatric disorders, including SZ (Goldman-Rakic and Selemon, 1997; Millan et al., 2012; Seamans and Yang, 2004). In fact, cognitive impairments, particularly working memory deficits, are considered to be a core feature of SZ. Therefore, it is possible that a loss of Akt contributes to PFC dysfunction. Indeed, deletion of Akt1 causes not only a decrease of dendritic architecture in the PFC, but also abnormal working memory performance (Lai et al., 2006). Notably, only under activation of D2 receptors (D2Rs) do Akt knockout mice display working memory deficits, indicating that Akt deficiency makes PFC dysfunction susceptible to tighter regulation by dopamine (DA) transmission (Lai et al., 2006). As a major neurotransmitter in the PFC, DA has long been implicated in SZ. Indeed, all antipsychotic drugs exert their actions by blocking D2Rs (Creese et al., 1976; Seeman and Lee, 1975; Seeman et al., 1976). Recent studies have shown that, apart from classical cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) and phospholipase C (PLC) signaling pathway (Greengard, 2001; Missale et al., 1998; Trantham-Davidson et al., 2004), D2Rs act through a cAMP-independent Akt–glycogen synthase kinase 3 (GSK-3) signaling cascade (Beaulieu et al., 2004, 2005). Activation of D2Rs allows β-arrestin 2 to bind with protein phosphatase 2 (PP2A) and Akt to form a complex in which PP2A dephosphorylates and deactivates Akt, resulting in activation of GSK-3 (Beaulieu et al., 2004, 2005). However, how Akt deficiency affects DA transmission and consequently results in abnormalities in PFC functioning remains unknown.

It is well established that alterations in gamma aminobutyric acid (GABA) receptor signaling is associated with SZ (Benes and Berretta, 2001; Lewis et al., 2005). The modulation of $GABA_AR$-mediated inhibitory transmission by DA is critical for normal cognitive processing. Furthermore, DA exhibits bidirectional effects on $GABA_AR$–mediated inhibitory postsynaptic currents (IPSCs); these currents are enhanced by activation of D1 receptors (D1Rs) and depressed by activation of D2Rs (Li et al., 2011, 2012; Seamans

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et al., 2001; Trantham-Davidson et al., 2004). Our recent findings suggest that activation of GSK-3β is involved in hyperdopamine/D2R-induced attenuation of $GABA_AR$–mediated IPSCs (Li et al., 2012). In this study, we further investigate whether and how Akt deficiency affects DA modulation of IPSCs in the PFC. To mimic cortical Akt deficiency, we blocked Akt activity by incubating PFC slices with Akt inhibitors. We found that disruption of Akt decreased DA sensitivity by increasing D2R internalization, which led to a significant change in DA modulation of IPSCs in the PFC.

2. Materials and methods

2.1. Animals

A total of 112 Sprague Dawley rat pups were used for this study. The pups on postnatal days 10 and their moms were purchased from the Charles River Laboratories (Wilmington, MA) and they were housed in the animal facility with at least two days of accommodation before being used for experiments. Among these animals, 95 were aged between P12-21 (before weaning) with the sex of these animals not identified, and 17 male animals between P22 to P30 used for electrophysiological recordings were also included. We did not observe significant differences between the young (P12-21) and older male animals (P22-30), so all electrophysiological data were pooled together, as we previously reported (Li et al., 2012). 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. Preparation of prefrontal cortical slices

The rats were anesthetized with Euthasol (0.2 ml/kg; Virbac AH), and the brains were immediately removed and placed in ice-cold (~4 °C) sucrose solution (in mM: 2.5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 0.5 CaCl2, 7.0 MgSO4, 213 sucrose, pH 7.4) buffered with 95% O2 and 5% CO2. Neocortex containing medial PFC (PrL) was horizontally sectioned at a thickness of 300 μm using a Leica MT1000 Vibratome (Leica Microsystems). Slices were then transferred to a holding chamber submerged in oxygenated artificial cerebrospinal fluid (ACSF) (in mM: 128 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 1 MgSO4, 26 NaHCO3, and 10 dextrose, pH 7.4.) at 35 °C for 1 h and then remained at room temperature until used for electrophysiology or Western blotting.

2.3. Electrophysiology

Whole-cell patch-clamp recordings were conducted in prefrontal cortical slices through an upright Zeiss Axioskop 2 microscope (Carl Zeiss) that is equipped with optics of infrared-differential interference contrast (IR-DIC) and a digital video camera system. The recordings were conducted at ~35 °C and the resistance of the recording pipette was 5–7 MΩ. The inhibitory postsynaptic currents (IPSCs) in layer 5 pyramidal neurons were elicited by stimulating layer 2/3 with either a single pulse or paired pulses at 10 Hz (0.1 ms, 10–100 μA, 10 s inter-stimulus interval) through a bipolar electrode. The miniature IPSCs (mIPSCs) and spontaneous IPSCs (sIPSCs) were recorded at −65 mV in the presence of AP5 (D-(−)-2-Amino-5-phosphonopentanoic acid; 50 μM) and DNQX (6,7-dinitroquinoxaline-2,3-dione; 20 μM) to block glutamate receptor mediated currents with or without TTX (tetrodotoxin; 0.5 μM), respectively. GABA-induced inward currents were recorded at −60 mV in the presence of AP5 (50 μM), DNQX (20 μM) and TTX (0.5 μM) by bath application of GABA (300 μM). A high chloride Cs + -based intracellular solution (134 mM CsCl2, 2 mM MgCl2, 2 mM Na2-ATP, 0.5 mM Na2GTP, 5 mM Na2-phosphocreatine, 1 mM EGTA, 10 mM HEPES, pH 7.25) was used for IPSC recordings. All neurons without stable baseline recording of IPSCs for 5 min or with series resistance increases of more than 20%, were discarded from further analysis. All drug effects were then normalized to baseline levels. Statistical analyses were performed using Student t-test between the controls and individual drug treatment groups and one-way or two-way analysis of variance (ANOVA) for multiple-comparisons among the several experimental groups. All data were presented as mean ± standard error.

2.4. Treatment of brain slices and protein extraction

Brain slices were incubated with oxygenated ACSF in the presence of different drugs for 15 min. PFC tissue was then dissected from drug-treated brain slices. For total protein extraction, the tissue was homogenized in RIPA buffer (50 mM Tris, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 20 mM β-glycerophosphate disodium salt hydrate, 1 mM Na3VO4, 1 mg/ml leupeptin, 0.1% SDS, 1% Triton, 1 mM phenylmethanesulfonylfluoride (PMSF), and 1 mM NaF) and then centrifuged at 10,000 g for 10 min at 4 °C. The supernatant fraction was aliquoted and stored at −80 °C. For synaptosomal membrane protein extraction, tissue was homogenized in sucrose buffer (4 mM HEPES pH7.4, 320 mM sucrose, 2 mM EGTA, 10 mM Na pyrophosphate, 1 mM Na Orthovanadate, 10 mM NaF, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 0.1 mM PMSF), and then centrifuged at 1000 g for 10 min to remove large cell fragments and nuclear material. The supernatant was centrifuged at 15,000 g for 15 min to yield cytoplasmic proteins in the supernatant. The pellet from this spin was resuspended in homogenization buffer and centrifuged at 15,000 g for an additional 15 min to yield washed synaptosomes. The synaptosomal fraction was then hypoosmotically lysed and centrifuged at 25,000 g for 30 min to yield synaptosomal plasma membranes in the pellet. Pellets were then resuspended in homogenization buffer and aliquoted and stored at −80 °C until further use.

2.5. Western blots

Equal amounts of proteins (10–20 μg) were running on a 7.5% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) gel. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA) and blocked with 5% nonfat milk. The membranes were then incubated with the following primary antibodies overnight at 4 °C: anti-D2R (1: 250, Santa Cruz Biotechnology, Dallas, TX), anti-β-arrestin2 (1:500, Cell Signaling Technology, Boston, MA), anti-clathrin (1:2000, Abcam, Cambridge, MA) and anti-actin (1: 100,000, Sigma-Aldrich, St. Louis, MO). After three 20 min washes, the membranes were incubated with HRP-conjugated goat anti-mouse or anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at 1:5000 for 2 h. The immunopositive protein bands were detected with ECL Western Blotting System (GE Healthcare Biosciences, Piscataway, NJ). After the exposure of membranes to HyBlot CL Autoradiography film (Denville Scientific Inc., Holliston, MA), the band densities were measured with NIH Image J software. Final data were normalized to actin. To minimize the interblot variability, each sample was run and analyzed four times. Statistical analyses were similarly performed using Student t-test between the controls and individual drug treatment groups and one-way ANOVA for multiple-comparisons among the several experimental groups. The data were presented as mean ± standard error.