Neurobiology of Disease 86 (2016) 109–120

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Wnt signaling pathway improves central inhibitory synaptic transmission in a mouse model of Duchenne muscular dystrophy

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ARTICLE INFO

Article history:
Received 5 June 2015
Revised 2 November 2015
Accepted 23 November 2015
Available online 2 December 2015

Keywords:
Dystrophin
mdx mice
Wnt signaling
GABA synapses

ABSTRACT

The dystrophin-associated glycoprotein complex (DGC) that connects the cytoskeleton, plasma membrane and the extracellular matrix has been related to the maintenance and stabilization of channels and synaptic receptors, which are both essential for synaptogenesis and synaptic transmission. The dystrophin-deficient (*mdx*) mouse model of Duchenne muscular dystrophy (DMD) exhibits a significant reduction in hippocampal GABA efficacy, which may underlie the altered synaptic function and abnormal hippocampal long-term plasticity exhibited by *mdx* mice. Emerging studies have implicated *Wnt* signaling in the modulation of synaptic efficacy, neuronal plasticity and cognitive function. We report here that the activation of the non-canonical *Wnt-5a* pathway and Andrographolide, improves hippocampal *mdx* GABAergic efficacy by increasing the number of inhibitory synapses and $GABA_A$ receptors or GABA release. These results indicate that *Wnt* signaling modulates GABA synaptic efficacy and could be a promising novel target for DMD cognitive therapy.

1. Introduction

Duchenne muscular dystrophy (DMD) is a lethal, X-linked recessive neuromuscular disease that is characterized by the deficiency of dystrophin, a gene on Xp21, which is responsible for the early onset of the genetic disease (Rodino-Klapac et al., 2013). Lack of dystrophin in the brain has been associated with impairments in behavioral and cognitive function (Anderson et al., 2002; Bresolin et al., 1994), mainly with impaired memory retention, procedural learning and spatial memory (Muntoni et al., 1991; Vaillend et al., 1995, 2004).

In the central nervous system (CNS), dystrophin is expressed in postsynaptic densities of hippocampal, amygdale and cortical neurons (Bies et al., 1992; Comim et al., 2011). It is localized on the inner-side of the plasma membrane as part of a protein complex called the dystrophin-associated glycoprotein complex (DGC) that connects the cytoskeleton, plasma membrane and the extracellular matrix (Blake and Kroger, 2000; Perronnet and Vaillend, 2010). DGC has been associated with the maintenance and stabilization of channels and receptors, which are both essential to synaptogenesis and synaptic transmission (Albrecht and Froehner, 2002; Haenggi and Fritschy, 2006; Perronnet and Vaillend, 2010; Waite et al., 2009).

The dystrophin-deficient (*mdx*) mouse model of DMD exhibits a significant reduction in the number and size of $GABA_A$ receptor ($GABA_A$-R) clusters in pyramidal neurons and Purkinje cells (Grady et al., 2006; Knuesel et al., 1999; Kueh et al., 2011) without a change in the total number of $GABA_A$-Rs (Kueh et al., 2008). In CA1 hippocampal synapses, long-term plasticity is abnormally enhanced. These deficits in GABA efficacy may underlie altered synaptic function and abnormal long-term potentiation (LTP) (Dallerac et al., 2011; Vaillend and Billard, 2002; Vaillend et al., 1999). The *Wnt* signaling pathway plays a key role in the neuronal development and maintenance of the nervous system by modulating synaptic structure and function (Inestrosa and Arenas, 2010; Oliva et al., 2013a; Rosso and Inestrosa, 2013; Salinas

Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANDRO, andrographolide; APV, 2-amino-5-phosphonovaleric acid; AraC, 1-β-D-arabinofuranosylcytosine; CaMKII, calmodulin-dependent protein kinase II; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DGC, dystrophin-associated glycoprotein complex; DMD, Duchenne muscular dystrophy; EPSC, excitatory postsynaptic currents; fIPSP, field inhibitory postsynaptic potentials; fEPSP, field excitatory postsynaptic potentials; Foxy-5, *Wnt-5a* analog; GABA, gamma-aminobutyric acid; $GABA_A$-R, $GABA_A$ receptor; IPSC, inhibitory postsynaptic current; JNK, c-Jun. NH2-terminal kinase; Mdx, dystrophin-deficient mouse; mEPSC, mini excitatory postsynaptic currents; mIPSC, mini inhibitory postsynaptic currents; NMDA, N-methyl D-aspartate receptor; PPR, paired pulse ratio; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TTX, tetrodotoxin.

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2015.11.018
0969-9961/

and Zou, 2008). Recently, it has been reported that *Wnt-5a* ligands, through the activation of a non-canonical pathway, modulate the excitatory and inhibitory transmission at the postsynaptic region (Cerpa et al., 2010; Cuitiño et al., 2010; Varela-Nallar et al., 2010; Vargas et al., 2014; Box 1). In inhibitory synapses, *Wnt-5a* enhances the amplitude of the inhibitory postsynaptic current (IPSC) through the insertion and clustering of $GABA_A$-Rs, increasing receptor recycling without affecting the endocytic process (Cuitiño et al., 2010). Together, these findings indicate that *Wnt-5a* increases the assembly of $GABA_A$-Rs receptors and modulates the synaptic plasticity of inhibitory circuits. Because of the essential role of the *Wnt* signaling pathway in $GABA_A$-R cell surface stability and activity, we hypothesize that the activation of the non-canonical *Wnt* pathway might improve the GABAergic deficit of the *mdx* mouse model of DMD. It has been suggested that andrographolide (ANDRO), the major constituent of *Andrographis paniculata*, has some protective properties in the brain. Recent results from our group suggest that ANDRO can protect against brain impairment during Alzheimer's disease progression (Serrano et al., 2014) and reduce the muscle impairment in *mdx* mice (Cabrera et al., 2014).

In the present study, we show that the activation of the non-canonical *Wnt-5a* pathway or ANDRO improve *mdx* hippocampal GABAergic efficacy by increasing the number of inhibitory synapses and $GABA_A$-Rs. These findings indicate that *Wnt* signaling modulates GABA synaptic efficacy and could be a promising novel target for *mdx* cognitive therapy.

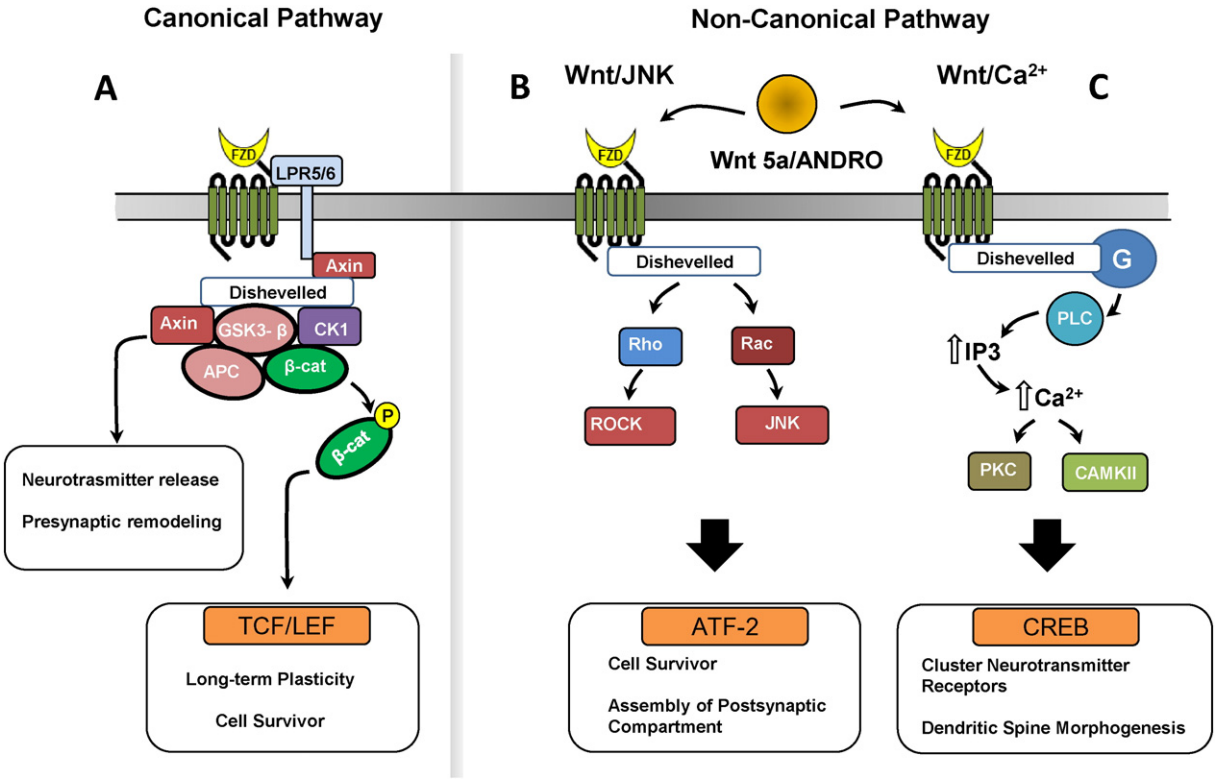
2. Materials and methods

2.1. Reagents and antibodies

The *Wnt-5a* analog, Foxy-5 (Safholm et al., 2006) was obtained from Genemed Synthesis (San Antonio, Texas), and ANDRO was obtained from Sigma-Aldrich (St. Louis, MO). The primary antibodies used were rabbit Anti-$GABA_A$ receptor (γ2 subunit, #G0545 Sigma), rabbit anti-SAPK/JNK (#9252 Cell Signaling, Danvers, MA), rabbit anti-phospho-SAPK/JNK (#9251S Cell Signaling), mouse anti-calcium calmodulin-dependent protein kinase II (CaMKII) (sc-32,288 Santa Cruz Biotechnology Inc., Dallas, TX) mouse anti-phospho-CaMKII (sc-32,289) and mouse anti-β-actin clone AC-15 (A1978 Sigma-Aldrich, St. Louis, MO).

2.2. Animals

Parental strains of wild-type (C57BL/10) and *mdx C57BL/10ScSn-Dmdmdx* (*mdx*) mice were obtained from Jackson Laboratories (BarHarbor, MEHight). The animals were kept at room temperature with a 24-h night–day cycle and fed pellets and water ad libitum. All protocols were conducted under strict accordance with and with the formal approval of the Animal Ethics Committee of the Pontificia Universidad Católica de Chile and Universidad de Valparaíso, Chile.



Box. 1. *Wnt* signaling pathways. (A) Activation of canonical *Wnt*/β-catenin dependent pathway starts with the binding of *Wnt* ligand to Frizzled (Fz) receptors and the co-receptor low-density lipoprotein receptor-related protein 5 (LRP5)/LRP6. Under these conditions GSK3β (glycogen synthase kinase-3β), is blocked and β-catenin accumulates in the cytoplasm before moving into the nucleus, where it activates the transcription of *Wnt* target genes mediated by TCF (T-cell factor)/LEF (lymphoid enhancer factor). Canonical *Wnt* pathway participates clustering of pre-synaptic proteins and the neurotransmitter release as well as in synaptic plasticity and cell survival (see Inestrosa and Arenas, 2010). (B) In the non-canonical *Wnt*/JNK (c-Jun. N-terminal kinase) pathway, activation of Dishevelled by the binding of *Wnt* to Fz, induces the activation of Rho and Rac small GTPases. Activation of ROCK signals through Rho and the activation of JNK signals through the Rac. This pathway is involved in the clustering of post-synaptic proteins and can also lead gene transcription mediated by ATF2 (activating transcription factor 2). (C) In non-canonical *Wnt*/Ca^{2+} signaling activation of Fz and Dishevelled (with *Wnt5a* or ANDRO) increases the intracellular Ca^{2+} concentration, which in turn activates both protein kinase C (PKC) and calcium/calmodulin-dependent protein kinase II (CaMKII); these kinases can then modify different signaling components. In both cases of non-canonical *Wnt* signaling, evidence suggests that G proteins are involved in the transduction of the *Wnt* signal. *Wnt*/Ca^{2+} pathway are involved in the clustering of post-synaptic proteins and the dendritic spine morphogenesis. (Modified from Oliva et al., 2013b and Inestrosa and Arenas, 2010).