Psychiatry Research 243 (2016) 210–218

Contents lists available at ScienceDirect

Psychiatry Research

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

Hippocampal and motor fronto-cortical neuroligin1 is increased in an animal model of depression

Pingfu Feng [a,b,*], Afaf A. Akladious [a], Yufen Hu [a,b]

[a] Louis Stokes Cleveland DVA Medical Center, Cleveland, OH, USA
[b] Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, Case Western Reserve University, Cleveland, OH, USA

ARTICLE INFO

Article history:
Received 13 January 2016
Received in revised form
9 May 2016
Accepted 26 June 2016
Available online 29 June 2016

Keywords:
Neuroligin
Animal model of depression
Wistar Kyoto rat
Immobility

ABSTRACT

Neuroligins (NLGNs) regulate synaptic excitability, neuronal signaling and sleep. We hypothesize that alteration of NLGNs is involved in the pathology of depression and tested the hypothesis in a model of depression using Wistar Kyoto (WKy) rat and its control, the Wistar (Wis) rat. We first evaluated behavioral deficits using the forced swim test and then characterized alterations of NLGN1 and NLGN2 with RT-PCR and Western Blotting in the prefrontal cortex, motor frontal cortex and hippocampus. Compared with controls of Wis rats, (1) the WKy rats had significantly shorter swim time and longer immobile time; (2) NLGN1 mRNA levels was higher in the motor frontal cortex and hippocampus in the WKy model; (3) NLGN1 protein was significantly higher in the motor frontal cortex, the prefrontal cortex and the hippocampus in the WKy model; (4) NLGN2 mRNA was significantly higher in the motor frontal cortex but significantly lower in the hippocampus in the WKy model. We concluded that NLGN1 gene and protein expression is higher in the motor frontal cortex, hippocampus and in the prefrontal cortex in the WKy rats suggesting that alterations of NLGN1 is involved in the pathology of depression but need to be further evaluated in human.

Published by Elsevier Ireland Ltd.

1. Introduction

Neuroligins (NLGNs) are a family of postsynaptic cell-adhesion proteins that regulate synaptic activity. NLGN, together with neurexin, a presynaptic cell-adhesion molecule, form transsynaptic complexes (Bottos et al., 2011; Mackowiak et al., 2014) and regulate synapse formation in the early stage of neuronal development and neuronal excitability in the later stages (Schapitz et al., 2010). NLGN is also believed to drive postsynaptic assembly through the binding of its intracellular domain to postsynaptic density protein 95 (PSD-95) (Shipman et al., 2011). NLGN has at least four isoforms: NLGN1, NLGN2, NLGN3, and NLGN4. NLGNs are known to enhance both excitatory and inhibitory synapse formation since individual NLGN isoforms preferentially localize to either glutamatergic or GABAergic synapses. PSD-95 is believed to be involved in the recruitment of NLGN1 to excitatory synapses (Giannone et al., 2013; Levinson et al., 2010). NLGN2 interacts with gephyrin, a GABAergic synapse-specific adhesion molecule, and exerts an inhibitory effect on synaptic excitability (Bang and Owczarek, 2013; Kasugai et al., 2010; Kohl et al., 2013). Studies in animals and humans indicated that NLGNs play an important role in regulating complex behaviors, possibly via their effects on synaptic excitability and plasticity (Mackowiak et al., 2014). NLGN1 knockout (KO) mice exhibit a significant impairment in spike timing-dependent long-term potentiation (LTP) of thalamic receptors (Jung et al., 2010) and reduced synaptic responses to the activation of glutamatergic perforant path granule cell inputs (Jedlicka et al., 2015), suggesting that NLGN1 can modulate synaptic plasticity via the regulation of NMDA receptors (Jung et al., 2010). These NLGN1 KO mice display lower response rate to forepaw electrical stimulation (Belanger-Nelson et al., 2015) and show deficits in spatial learning and memory that correlate with impaired hippocampal LTP, a dramatic increase in repetitive, stereotyped grooming (Blundell et al., 2010) and a subset of sensory behaviors and sensory processing that are strikingly similar to traits frequently associated with autism spectrum disorders (Hunter et al., 2010). Deletion of NLGN2 increases the excitability of hippocampal cortical neurons, via impaired $GABA_A$ receptor clustering (Jedlicka et al., 2011). Conditional NLGN2 KO in mice induced profound reductions in synaptic inhibition, reduced behavioral stimulation of immediate-early gene expression in the prefrontal cortex and produced impairments in anxiety, fear memory and social interaction behaviors (Liang et al., 2015). The NLGN3 and NLGN4 isoforms have also been implicated in autism (Hill-Yardin et al., 2015; Yu et al., 2011) and schizophrenia (Fujita-

* Corresponding author. Current address: Louis Stokes Cleveland DVA Medical Center Research, Room K205, 10701 East BLVD, Cleveland, OH 44106, USA.
E-mail address: dxpfeng@gmail.com (P. Feng).

http://dx.doi.org/10.1016/j.psychres.2016.06.052
0165-1781/Published by Elsevier Ireland Ltd.

Jimbo et al., 2015). Taken together, these data implicate the NLGN family in neuronal excitability, neuronal activity and behavioral function.

Major depressive disorders (MDD) represents a commonly occurring group of mental disorders in the general population (Kessler et al., 2011) and selective serotonin (5-hydroxytryptamine, 5-HT) reuptake inhibitors (SSRIs) remain the front line of treatment for this group of disorders (Dale et al., 2015; Zhang et al., 2015). Scientific features from several studies have suggested that NLGN1 may be involved in the pathology of MDD. First, impaired cortical excitability has been observed following post-exercise facilitation testing in patients with MDD (Reid et al., 2002; Samii et al., 1996; Shajahan et al., 1999). Impaired cortical excitability has also been reported to be associated with changes in core symptomatology including loss of interest and motivation consistent with the findings of increased cortical inhibition in depression (Steele et al., 2000). Moreover, a genomic clinical study comparing subjects with MDD to those without psychiatric disorders has demonstrated a single nucleotide polymorphism near the region encoding NLGN1 on chromosome 3 (Lewis et al., 2010). Furthermore, the mood stabilizer lithium has been recently shown to suppress NLGN1 levels (Park et al., 2015), while NLGN1 has been shown to be increased after sleep deprivation (Massart et al., 2014), a factor that is closely involved in MDD, since sleep loss or insomnia is common in MDD (Gold, 2015; Porkka-Heiskanen et al., 2013). Taken together, these data suggest that NLGN1 may be associated with pathology of depression. To further test this hypothesis, we utilized the Wistar-Kyoto (WKy) model, a well-characterized and continuously used rat model of depression (Marcinkiewcz and Devine, 2015; Nagasawa et al., 2015b; Pare, 1989b, 1992), that is associated with depressive behaviors including a tendency for the animal to float in the water instead of swimming in an attempt to escape, inactivity in open-field tests, and cognitive impairment (Lahmame et al., 1997; Pare, 1992; Will et al., 2003).

2. Materials and methods

2.1. Experimental design and animals

In order to reduce individual variation due to sex hormones during different stages of the menstrual cycle, only male rats were used in the study. Ten week-old of Wistar (Wis) rats (n=11) and WKy rats (n=11) were purchased (Harlan, Inc., Indianapolis, Indiana). All animals were housed in the Animal Resources Facility of the Louis Stokes Cleveland DVA Medical Center (LSCDVAMC) under standard group housing conditions and allowed a two-week acclimation period following arrival. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the LSCDVAMC. Behavioral testing was performed when the animals were between 3 and 4 months old. Two days after completion of behavioral testing, animals were sacrificed under CO_2 anesthesia, and prefrontal cortex (PFC), motor frontal cortex (MFC) and hippocampus were dissected according to the mouse brain atlas (Paxinos and Franklin, 2001). Dissection of PFC included cingulate cortex, prelimbic cortex, infralimbic cortex and medial orbital cortex; MFC primarily included primary motor cortex (M1) and secondary motor cortex (M2), according to a recent review (Bicks et al., 2015). All tissue was subjected to protein and RNA extraction.

2.2. Forced swim tests

The light/dark schedule was established as lights on at 8:00 p.m. and off at 8:00 a.m. at least one week before testing was initiated. All tests were conducted between 9:00 a.m. and 12:00 noon, i.e., during the dark phase, under dim red light. On each testing day, an identical number of animals were chosen from each group for evaluation. The forced swim test was conducted with a modified method according to our previously published studies (Feng et al., 2008, 2007). Briefly, a plastic cylinder with a 12-in. outside diameter and 24 in. height was used. The tank was then filled with water (28 °C) up to 18 in. Before the test, animals were housed in their home cages in a quiet testing room and a video recorder was started for recording. The subject was then rapidly placed into the test apparatus and swimming behavior was recorded over 10 min. Data was played back for offline scoring. Swim and immobility times were scored separately in 5-min intervals.

2.3. RNA extraction and RT-PCR

Bilateral brain regions including left and right prefrontal cortex, frontal (motor) cortex and hippocampi were dissected after decapitation under deep anesthesia. Tissue dissected from one side of the rat brain was treated with RNAse-free DNAse (Ambion, Austin, TX) and used for total cellular RNA extraction with Trizol (Invitrogen, Grand Island, NY) according to the Manufacturer's instructions. First-strand cDNA synthesis was generated from 3 μg of total RNA in a 20-μl volume using random primers (Invitrogen) containing 200 units of M-MLV reverse transcriptase (Invitrogen).

Dissected brain tissue was treated with TRIzol (1 ml TRIzol per 50–100 mg tissue) and homogenized using a Power Gen 500 homogenizer (Fisher Scientific, Pittsburgh, PA). Tissue extracts were then mixed with 0.2 ml chloroform, inverted and incubated at room temperature for 2–3 min. Samples were then centrifuged at 12,000 rpm for 15 min at 4 °C and the upper aqueous RNA portions were transferred to new tubes. Total RNA was precipitated with 0.5 ml Isopropyl Alcohol at −80 °C overnight, centrifuged at 12,000 rpm for 10 min at 4 °C, washed using 0.5 ml 75% ethanol and centrifuged again at 12,000 rpm for 10 min at 4 °C. Sample tubes were air dried for 5–10 min after discarding the supernatant. RNAs were dissolved in nuclease free H_2O and reverse transcribed following the Manufacturer's instruction (Invitrogen).

Expression levels of NLGN1 and NLGN2 were quantified by relative quantitative RT-PCR using QuantumRNA 18S Internal Standards kits (Ambion, Austin, TX). Relative quantitative PCR was performed by multiplexing corresponding primers (NLGN-1 forward 5′-GGGGATGAGGTTCCCTATGT-3′, reverse 5′-GTGGGAAAGGCTGATGTGAC-3′; NLGN-2 forward 5′- CTGCCCTACGTCTTTGGTGT-3′, reverse 5′-AGCTCTGTGTGCAGGTTGTG-3′), 18S primers and competimers. Linear range for NLGN1 and NLGN2 amplicons were determined to be between 27–35 cycles and 21–31 cycles, respectively. The optimal ratio of 18S primers to competimers was 1:19 for both amplicons. PCR was performed at 94 °C denaturation for 50 s, 58 °C (NLGN1) or 60 °C (NLGN2) annealing for 50 s and 72 °C elongation for 50 s for 32 (NLGN1) or 27 (NLGN2) cycles. The PCR product was electrophoresed on polyacrylamide gel and stained with CYBR Gold (Molecular Probes, Eugene, OR). Gels were imaged using a FotoDyne gel documentation system (FOTODYNE Incorporated, Hartland, WI). Individual band intensity was quantified using Image J software (NIH). The relative values of NLGNs were derived by dividing the signal obtained for NLGN by that obtained for 18S.

2.4. Protein extraction and Western Blotting

Tissues dissected from the contralateral (remaining) hemisphere of the rat brain were suspended in ice cold RIPA lysis buffer with PMSF, Aprotinin and Na_3VO_4, and homogenized on ice using an ultrasonic Dismemberrator (Fisher Scientific, Inc. Pittsburgh, PA). Tissues were lysed on ice for 30 min, vortexed and centrifuged