Molecular Psychiatry
https://doi.org/10.1038/s41380-019-0371-y

ARTICLE

Ahnak scaffolds p11/Anxa2 complex and L-type voltage-gated calcium channel and modulates depressive behavior

Junghee Jin[1] · Dionnet L. Bhatti[1] · Ko-Woon Lee[1] · Lucian Medrihan[1] · Jia Cheng[1] · Jing Wei[2] · Ping Zhong[2] · Zhen Yan[2] · Cassandra Kooiker[1] · Claire Song[1] · Jung-Hyuck Ahn[3] · Gerald J. Obermair[4] · Amy Lee[5] · Jodi Gresack[1] · Paul Greengard[1] · Yong Kim[1]

Received: 30 August 2018 / Revised: 14 December 2018 / Accepted: 11 January 2019
© The Author(s) 2019. This article is published with open access

Abstract

Genetic polymorphisms of the L-type voltage-gated calcium channel (VGCC) are associated with psychiatric disorders including major depressive disorder. Alterations of S100A10 (p11) level are also implicated in the etiology of major depressive disorder. However, the existence of an endogenous regulator in the brain regulating p11, L-type VGCC, and depressive behavior has not been known. Here we report that Ahnak, whose function in the brain has been obscure, stabilizes p11 and Anxa2 proteins in the hippocampus and prefrontal cortex in the rodent brain. Protein levels of Ahnak, p11, and Anxa2 are highly and positively correlated in the brain. Together these data suggest the existence of an Ahnak/p11/Anxa2 protein complex. Ahnak is expressed in p11-positive as well as p11-negative neurons. Ahnak, through its N-terminal region, scaffolds the L-type pore-forming α1 subunit and, through its C-terminal region, scaffolds the β subunit of VGCC and the p11/Anxa2 complex. Cell surface expression of the α1 subunits and L-type calcium current are significantly reduced in primary cultures of Ahnak knockout (KO) neurons compared to wild-type controls. A decrease in the L-type calcium influx is observed in both glutamatergic neurons and parvalbumin (PV) GABAergic interneurons of Ahnak KO mice. Constitutive Ahnak KO mice or forebrain glutamatergic neuron-selective Ahnak KO mice display a depression-like behavioral phenotype similar to that of constitutive p11 KO mice. In contrast, PV interneuron-selective Ahnak KO mice display an antidepressant-like behavioral phenotype. Our results demonstrate L-type VGCC as an effector of the Ahnak/p11/Anxa2 complex, revealing a novel molecular connection involved in the control of depressive behavior.

Introduction

S100A10 (p11) is a member of the S100 protein family [1]. Alterations of p11 are implicated in the etiology of major depressive disorder (MDD) and in the therapeutic actions of antidepressants [2]. The levels of p11 mRNA and protein in the brain are downregulated in depressed humans, suicide victims, and a mouse model of depression [3–5], suggesting an important role for p11 in depression pathophysiology [5]. p11 null mice exhibit depression-like behaviors and abolished behavioral responses to antidepressants [3, 6]. Conversely, p11 overexpression in mice leads to an antidepressant-like behavioral phenotype [3]. Because p11 is an adaptor-like small protein with a molecular weight of 11 kDa, its function is likely mediated by its interacting partners. Thus, it is critical to identify binding partners and downstream effectors of p11 and characterize their role in depression-like behaviors in order to fully understand the mechanism by which p11 controls depression-like behaviors.

Supplementary information The online version of this article (https://doi.org/10.1038/s41380-019-0371-y) contains supplementary material, which is available to authorized users.

✉ Yong Kim
kimyo@rockefeller.edu

[1] Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

[2] Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, State University of New York, Buffalo, NY, USA

[3] Department of Biochemistry, Ewha Womans University, Seoul, South Korea

[4] Division of Physiology, Medical University Innsbruck, 6020 Innsbruck, Austria

[5] Department of Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA, USA

Published online: 13 February 2019

In an initial study in our laboratory, p11 was identified as a binding partner of several subtypes of serotonin receptors such as 5HT1B, 5HT1D, and 5HT4 by yeast two-hybrid assays [3, 7]. p11 increases the surface expression of 5HT1B and 5HT4, thereby potentiating serotonergic signaling and facilitating the actions of antidepressants such as selective serotonin reuptake inhibitors (SSRIs) [2, 3, 7]. p11 forms a heterotetrameric protein complex with Anxa2 [8]. We identified a chromatin-remodeling factor, named SMARCA3, as a binding partner of the p11/Anxa2 complex from HEK293 cells [9]. SMARCA3 constitutive knockout (KO) did not cause depression-like behaviors but it abolished behavioral and neurogenic responses to SSRIs [9]. Using this binding assay, we also identified Ahnak as a binding partner of the p11/Anxa2 complex [9]. Ahnak is an extremely large protein with a molecular weight of 680 kDa [10, 11]. The interaction of Ahnak with the p11/Anxa2 protein complex was first demonstrated in a canine kidney cell line Madin-Darby Canine Kidney (MDCK), in which p11 and Anxa2 were required for recruitment of Ahnak to the plasma membrane [12]. Previous reports showed Ahnak expression in endothelial cells in the blood brain barrier, epithelial cells in the choroid plexus and ependymal cells in the ventricular wall of the adult mouse brain [13, 14], in which a role for Ahnak in the formation of tight junctions was proposed [13]. However, the neuronal function of Ahnak and the functional significance of its interaction with the p11/Anxa2 complex in the brain have not yet been investigated.

L-type voltage-gated calcium channels (VGCCs) are heteromultimeric protein complexes composed of a pore-forming α1 subunit and two auxiliary subunits: cytoplasmic β subunit and extracellular α_2/δ subunit [15]. Two L-type α1 subunits ($Ca_v1.2$ and $Ca_v1.3$) are expressed in the brain, and L-type VGCCs are mainly localized in the soma and dendrites of neurons. Voltage-dependent L-type channel opening and calcium influx initiate both CaMKIV and MAPK signaling pathways, eventually leading to activation of CREB-dependent gene transcription [16–18]. Because L-type VGCC-mediated calcium signaling and gene regulation contribute to neuronal development, synaptic plasticity, and homeostatic control of neuronal circuitry [19, 20], alterations of the level or activity of L-type VGCC may result in behavioral abnormalities. In fact, previous genome-wide association studies have shown that $Ca_v1.2$ gene (CACNA1C) polymorphisms are associated with MDD, bipolar disorder, and schizophrenia [21–23]. Alterations of L-type channels have emerged as susceptibility factors for multiple psychiatric disorders [21, 24, 25]. Intriguingly, Ahnak was known as a binding partner of the β subunit of cardiac VGCC [26, 27] and the regulation of L-type VGCCs by Ahnak has been reported in cardiomyocytes [26–28], osteoblasts [29], and T cells [30], implicating Ahnak in the regulation of neuronal L-type VGCC.

In the present study, we show that Ahnak is a stabilizer of the p11/Anxa2 protein complex and a pivotal regulator of L-type VGCCs in the brain. In addition, our results indicate that neuronal type-selective Ahnak KO mice display modulated depressive behavior, elucidating a neuronal function of Ahnak potentially relevant to the pathophysiology of psychiatric disorders.

Materials and methods

Animals

All procedures for biochemical and behavioral experiments involving animals were approved by The Rockefeller University Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health guidelines. p11 KO mice were generated previously [3]. Ahnak KO mice [31] were received from RIKEN Bio Resource Center. p11-EGFP (HC85) mice were obtained from GENSAT (www.gensat.org). Floxed Ahnak mice were generated in Taconic-Artemis (Germany) and maintained at The Rockefeller University. EMX-Cre (stock 005628) and PV-Cre (stock 008069) lines were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). We produced the progeny of the Ahnak KO lines by in vitro fertilization (IVF) and embryo transfer techniques (Transgenic facility, The Rockefeller University) to provide a sufficient number of animals of the same age for the behavioral tests. All mice are of C57BL/6 background. Sixteen-week-old male mice were used for behavioral tests. 6-8 weeks old male mice were used for whole-cell patch-clamp recordings. Timed-pregnant female mice (E17) were used for primary cortical neuronal cultures. Mice were housed 2–5 per cage with a 12:12-h light/dark cycle and ad libitum access to food and water. Mice were assigned to experimental groups based on their genotype. Selection of animal samples out of different experimental groups for electrophysiology and biochemical analyses was performed randomly in a blinded fashion.

Pulldown assay

GST pulldown assay was performed as described previously [9]. Rat forebrain was homogenized with homogenization buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 2 mM $MgCl_2$ supplemented with 1% Triton X-100 and a protease inhibitor cocktail (cOmplete, Sigma-Aldrich). The soluble fraction was incubated with GST, GST-p11, or GST-p11/Anxa2 hybrid immobilized on glutathione–agarose beads (GE healthcare). After washing out the unbound

proteins, bound proteins were subjected to SDS-PAGE using 4-20% Tris-Glycine gel (Thermo Fisher Scientific, Grand Island, NY, USA). After protein staining with Coomassie Brilliant Blue R-250, the identity of the protein band specifically co-isolated with the p11/Anxa2 hybrid was determined by mass spectrometry (Yale/NIDA Neuroproteomics Center, New Haven, CT, USA).

Plasmid constructs

Plasmids expressing HA-$Ca_v1.2$ (sHA-$Ca_v1.2$) [32], HA-$Ca_v1.3$ (sHA-$Ca_v1.3a$) [33], or $_{4b}$ subunit (pA-$_{4b}$-V5) [34] were reported previously. The cDNAs of the N-terminal region (amino acids 1-298) and repetitive elements in the central region of human Ahnak (amino acids 1068-1579) were obtained from Pet28a-AHNAK-N-HIS-T7 and Pet28a-AHNAK-R-HIS-T7 as reported previously [35] and subcloned into the BamHI-XhoI site of a pAAV-CBA vector. The cDNA of the C-terminal region of mouse Ahnak (amino acids 3925-5656) [36] was cloned into the BamHI-EcoRI site of a pAAV-CBA vector. pAAV-CBA-Ahnak-N-Strep, pAAV-CBA-Ahnak-R-Strep, and pAAV-CBA-Ahnak-C-Strep were confirmed by sequencing.

Quantitative PCR (qPCR)

Total RNA was extracted from PFC and hippocampus using the RNeasy Mini kit (QIAGEN) according to the manufacturer's protocol. RNA concentration was measured by a Nanodrop 1000 spectrophotometer (Marshall Scientific, Hampton, New Hampshire, USA). Reverse transcription was performed with 1 g of total RNA using a High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. The qPCR was performed in a 20 reaction mixture containing 1 (10-50 ng) cDNA, 10 l SYBR Premix EX Taq (Takara Bio, Kusatsu, Shiga Prefecture, Japan), 0.4 Rox reference dye (50× , Takara Bio), and 200 nM of primers for each gene using the 7500 fast real-time PCR system (Thermo Fisher Scientific). The primer sequences were as follows: p11 (forward), 5 TGGAAACC ATGATGCTTACGTT-3; p11 (reverse), 5 GAAGCCCA CTTTGCCATCTC-3; AnxA2 (forward), 5- ATGTCTAC TGTCCACGAAATCCT-3; AnxA2 (reverse), 5 CGAA GTTGGTGTAGGGTTTGACT-3; GAPDH (forward), 5- AGGTCGGTGTGAACGGATTTG-3; GAPDH (reverse), 5 - TGTAGACCATGTAGTTGAGGTCA -3. The reaction ran at 95 °C for 30 s, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s and a dissociation cycle of 95 °C for 15 s, 60 °C for 60 s and 95 °C for 15 s. All PCRs were performed in triplicates and the specificity of the reaction was detected by melting curve analysis at the dissociation stage. Comparative quantification of each target gene was performed based on cycle threshold normalized to GAPDH using the CT method [37].

Immunoblotting and antibodies

Mouse prefrontal cortex (PFC) or hippocampal tissues were lysed with a lysis buffer (Pierce IP Lysis Buffer, 87788, Thermo Fisher Scientific) supplemented with a protease and phosphatase inhibitor cocktail (78442, Thermo Fisher Scientific). The tissue lysates were homogenized with a Dounce homogenizer (10 strokes) and centrifuged at 800 for 5 min. Protein levels in the supernatant were measured by the BCA method. The samples were mixed with the standard protein sample buffer, and subjected to SDS-PAGE with 4-20% Novex Tris-Glycine gels (Thermo Fisher Scientific) followed by protein transfer onto a nitrocellulose membrane. Immunoblotting was performed with a standard protocol using the following antibodies: anti-Ahnak (M-DY pAb [38], rabbit polyclonal, 1:50,000 or RU2064, rabbit polyclonal, 1:10,000), anti-p11 (goat polyclonal, AF2377, R&D systems, 1:1,000), anti-Anxa2 (mouse monoclonal, sc-28385, Santa Cruz, 1:1000), anti-Gapdh (mouse monoclonal, MAB374, Millipore Sigma, 1:5,000), anti-$Ca_v1.2$ (rabbit polyclonal, AB5156, EMD Millipore, 1:2,500), anti-$Ca_v1.3$ (rabbit polyclonal, Ab14436 [39], 1:1000), anti-Flag (mouse monoclonal, M2, Sigma, 1:1,000), anti-V5 (mouse monoclonal, SV5-Pk1, Abcam, 1:1000), anti-HA (rat monoclonal, 3F10, Sigma, 1:1,000), anti-$_1$ subunit (mouse monoclonal, 73052, NeuroMab, 1:1000), anti-$_{2a}$ subunit (rabbit polyclonal antibody, RU2077, 1:1,000), anti-$_3$ subunit (rabbit polyclonal antibody, RU2080, 1:1,000), anti-$_4$ subunit (mouse monoclonal, 75354, NeuroMab, 1:1000), anti-

subunit (mouse monoclonal, 73052, NeuroMab, 1:1000), anti-$_{2a}$ subunit (rabbit polyclonal antibody, RU2077, 1:1,000), anti-$_3$ subunit (rabbit polyclonal antibody, RU2080, 1:1,000), anti-$_4$ subunit (mouse monoclonal, 75354, NeuroMab, 1:1000), anti-Flag (mouse monoclonal, M2, Sigma, 1:1,000), anti-V5 (mouse monoclonal, SV5-Pk1, Abcam, 1:1000), anti-HA (rat monoclonal, 3F10, Sigma, 1:1,000), anti-