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Research Paper

Exogenous Alpha-Synuclein Alters Pre- and Post-Synaptic Activity by Fragmenting Lipid Rafts

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ABSTRACT

Alpha-synuclein (αSyn) interferes with multiple steps of synaptic activity at pre-and post-synaptic terminals, however the mechanism/s by which αSyn alters neurotransmitter release and synaptic potentiation is unclear. By atomic force microscopy we show that human αSyn, when incubated with reconstituted membrane bilayer, induces lipid rafts' fragmentation. As a consequence, ion channels and receptors are displaced from lipid rafts with consequent changes in their activity. The enhanced calcium entry leads to acute mobilization of synaptic vesicles, and exhaustion of neurotransmission at later stages. At the post-synaptic terminal, an acute increase in glutamatergic transmission, with increased density of PSD-95 puncta, is followed by disruption of the interaction between N-methyl-D-aspartate receptor (NMDAR) and PSD-95 with ensuing decrease of long term potentiation. While cholesterol loading prevents the acute effect of αSyn at the presynapse; inhibition of casein kinase 2, which appears activated by reduction of cholesterol, restores the correct localization and clustering of NMDARs.

1. Introduction

Alpha-synuclein (αSyn) a small cytosolic protein specifically enriched in the presynaptic nerve terminals (Maroteaux et al., 1988), was found as a major component of intraneuronal inclusion present in the brain of Parkinson's disease (PD) patients (Spillantini et al., 1997). Moreover, multiplication of αSyn gene, and genetic variations in the promoter region leading to increased expression of αSyn, cause familial and sporadic PD (Simon-Sanchez et al., 2009; Singleton et al., 2003). αSyn physiological role is still unclear, but various evidence suggests αSyn involvement in the modulation of distinct steps of the synaptic vesicle (SV) cycle (Lykkebo and Jensen, 2002). αSyn has been considered for long time exclusively an intracellular protein, but its identification in the cerebrospinal fluid and blood plasma (Borghi et al., 2000), suggested a role for extracellular αSyn in the spreading of neurodegeneration (Desplats et al., 2009). Oligomeric and fibrillar αSyn species have been identified as responsible for the toxicity and the spread of disease (Winner et al., 2011), also, a very recent work showed that injection of a non-amyloidogenic, truncated form of αSyn, was able to induce neuronal pathology, pointing to the importance of high dosage of soluble αSyn in the onset of the disease (Sacino et al., 2013). It is now believed that the balance between tetrameric and monomeric forms is important for the correct physiological activity of αSyn (Dettmer et al., 2016).

It is known that αSyn binds phospholipidic membranes, associating with specific microdomains, the lipid rafts (Fortin et al., 2004). At the synapse, receptors and ion channels have a precise localization in the plasma membrane (PM). One of the first proteins that localizes and clusters at synapses during the development is PSD-95, which is lipid raft-anchored (Perez and Bredt, 1998), and functions as multivalent synaptic scaffolding protein. The PDZ domain of PSD-95 was shown to bind the C-terminal tail of N-methyl-D-aspartate receptor (NMDAR) type-2 subunit (NR2b), and this binding is required for the correct localization of the receptor (Kornau et al., 1995).

In the striatum of parkinsonian animals, the localization of NMDAR subunits at synaptic sites is decreased, as is the localization of PSD-95

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in the synaptic membrane (Nash et al., 2005). NR2b subunits have been found to be specifically reduced in the dopamine denervated striatum of various animal PD models (Gardoni et al., 2006; Hallett et al., 2005). Subunit- and residue-specific phosphorylation differently affects the recruitment of synaptic and extrasynaptic NMDARs (Goebel-Goody et al., 2009). NMDAR binding to PSD95 is decreased by phosphorylation of the NR2b subunit at Serine 1480 (S1480) by casein kinase II (CK2) (Chen and Roche, 2007), as is NR2b surface expression (Chung et al., 2004). In electrophysiological studies, an increased channel gating upon application of purified CK2 has been however observed (Lieberman and Mody, 1999), suggesting a complex and not yet clear regulatory role of CK2. PD progression may also be consequent to molecular defects present at the pre-synaptic site, and indeed alterations in neurotransmitter release, SV trafficking (Murphy et al., 2000), and presynaptic plasticity (Goldberg et al., 2005) are caused by mutation or deletion of various genes involved in PD.

Recently it was demonstrated that application of monomeric extracellular αSyn may perturb calcium homeostasis through alteration of the fluidity of membranes (Melachroinou et al., 2013) or through re-localization of pre-synaptic $Ca_V2.2$ calcium channels in cholesterol-poor areas of the PM (Ronzitti et al., 2014). In rat hippocampal slices exposed to αSyn oligomers, the glutamatergic synaptic transmission was altered with impairment of further potentiation by physiological stimuli (Diogenes et al., 2012). However, the alterations caused by the accumulation of secreted monomeric αSyn, and the mechanisms through which extracellular αSyn contributes to neuronal dysfunction are largely unknown. Here we provide evidence that extracellular monomeric αSyn induces fragmentation of lipid rafts, thus altering raft-partitioning of several membrane-associated proteins. The detachment of proteins from lipid rafts brings about distinct effects in the short and long term exposure to αSyn, increasing basal and evoked calcium entry, neurotransmitter release and post-synaptic activation, and inducing, at later time points, SV exhaustion, defects in the proper clustering of the post-synaptic density (PSD) and impairment of long-term potentiation (LTP). These effects are prevented by cholesterol-loading and CK2 inhibition, respectively.

2. Materials & methods

2.1. Ethics statement

All the experimental procedures followed the guidelines established by the Italian Council on Animal Care and were approved by the Italian Government decree No. 27/2010.

2.2. Reagents

All chemicals, unless otherwise stated were purchased from Sigma-Aldrich (St. Louis, MO). The following primary antibodies were used: anti-PSD95 (Santa Cruz Biotechnology, Dallas, TX, catalog #SC-32290), anti-GAPDH (Santa Cruz Biotechnology, Dallas, TX, catalog #SC-25778) anti-flotillin1 (BD Biosciences, San Jose, CA, catalog #610821), anti-caveolin1 (BD Biosciences, San Jose, CA, catalog #610059), anti-α-synuclein (BD Biosciences, San Jose, CA, catalog #610787), anti-synaptophysin (Synaptic Systems, Goettingen, Germany, catalog #101011), anti-Homer (Synaptic Systems, Goettingen, Germany, catalog #160003), anti-synaptotagmin-1 (Synaptic Systems, Goettingen, Germany, catalog #105011) anti-NMDAR2a (Abcam plc, Cambridge, UK, catalog #ab133265), anti-NMDAR2b (Abcam plc, Cambridge, UK, catalog #ab28373), anti-NMDAR2b phospho S1480 (Abcam plc, Cambridge, UK, catalog #ab73014), anti-PKA (Santa Cruz Biotechnology, #sc-390548), and anti-GluA1 (Abcam plc, Cambridge, UK, catalog #ab32436). Syn peptides (amino acids 12–23 and 34–45) were obtained from Primmbiotech.

2.3. Expression plasmids

GFP-E-Syt1 was a kind gift from Pietro De Camilli (Yale School of Medicine, New Haven, CT). VAMP2-GFP was a kind gift from Flavia Valtorta (San Raffaele Scientific Institute, Milan, Italy). PSD-95-RFP was a gift from Johannes Hell (Addgene, Cambridge, MA, plasmid #52671).

αSynS129E-GFP was generated from αSyn-GFP (a gift from David Rubinsztein, Addgene, Cambridge, MA, plasmid #40822) with GeneArt™ Site-Directed Mutagenesis System (Life Technologies, Paisley, UK).

2.4. Neuronal culture

Primary cultures were obtained from hippocampi or cortices of C57BL6J mice (Harlan Laboratories Inc., Indianapolis, IN, USA) at embryonic day 18 (E18). Embryos were removed and dissected under sterile conditions. Cortices and hippocampi were dissociated by enzymatic digestion in 0.125% trypsin-EDTA (Life Technologies, Paisley, UK) for 30 min at 37 °C and 0.25 mg/ml DNase in Hank's Balanced Saline Solution (Life Technologies), 2 mM calcium chloride (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 37 °C. Trypsin activity was blocked by adding complete medium which consists of Neurobasal Media (Life Technologies) supplemented with 2% B27 (Life Technologies), 2 mM GlutaMax (Life Technologies), 100 U/ml penicillin-streptomycin (Life Technologies), and 10% fetal bovine serum (Life Technologies). After trypsinization, hippocampi and cortices were rinsed in complete medium without FBS, dissociated with a plastic pipette, and 20,000–40,000 hippocampal or cortical neurons were plated at a concentration of $0.20–1 \times 10^5$ cells/ml onto 18 mm or 25 mm diameter coverslips pre-coated with 0.1 mg/ml poly-D-lysine.

For LTP experiments primary cultures were obtained from hippocampi of E18 Sprague Dawley rats (Charles River). Dissociated cells were plated onto coverslips coated with poly-L-lysine at a density of 400 cells/mm^2. The cells were maintained in Neurobasal (Invitrogen, San Diego, CA) with B27 supplement and antibiotics, 2 mM glutamine, and 10 mM glutamate.

2.5. Electroporation and transfection of primary hippocampal and cortical neurons

For experiments with VAMP2 and E-Syt1, neurons (1×10^6) were electroporated with 1 μg of DNA using P3 Primary Cell 4D-Nucleofector Kit and the pulse code CU133 on the 4D-Nucleofector System (Lonza Group Ltd., Basel, Switzerland). For experiments with PSD-95, neurons were transfected using Lipofectamine 2000 (Life Technologies) at DIV 17–18. Cultures were analyzed at DIV 20–21.

2.6. Brain slices preparation

20-day old mice (C57BL/6JRccHsd or C57BL/6S, Harlan, Udine, Italy) were anesthetized with isofluorane and decapitated. Brains in cold carboxygenated artificial cerebrospinal fluid (ACSF, 124 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.1 mM $NaH2PO_4$, 2 mM $MgSO_4$, and 10 mM D-glucose), equilibrated with 95% O_2 and 5% CO_2 to yield pH 7.4, were sectioned on a vibrating microtome at a thickness of 100–300 μm.

2.7. αSyn purification

The construct encoding the full-length human wild-type αSyn inserted in the pET21d plasmid was a kind gift from Brett Lauring (Columbia University, New York, NY). Purification was performed as described previously (Martinez et al., 2003). Bacteria were induced during the exponential phase with 1 mM isopropyl β-D-1-thiogalactopyranoside for 2 h and harvested by centrifugation. The pellet was solubilized in 20 mM HEPES/KOH, pH 7.2, and 100 mM KCl (buffer A) and heated