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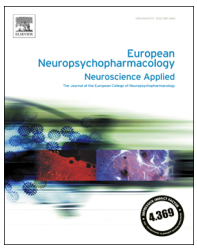

Interaction of NOS1AP with the NOS-I PDZ domain: Implications for schizophrenia-related alterations in dendritic morphology

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Abstract
Schizophrenia involves morphological brain changes, including changes in synaptic plasticity and altered dendritic development. Amongst the most promising candidate molecules for schizophrenia are neuronal nitric oxide (NO) synthase (NOS-I, also known as nNOS) and its adapter protein NOS1AP (previously named CAPON). However, the precise molecular mechanisms by which NOS-I and NOS1AP affect disease pathology remain to be resolved. Interestingly, overexpression of NOS1AP affects dendritic morphology, possibly through increased association with the NOS-I PDZ domain. To investigate the effect of NOS1AP on dendritic morphology we overexpressed different NOS1AP isoforms, NOS1AP deletion mutants and the aminoterminal 133 amino acids of NOS-I (NOS-I_{N133}) containing an extended PDZ domain. We examined the interaction of the overexpressed constructs with endogenous NOS-I by co-immunoprecipitation and the consequences of increased NOS-I/NOS1AP PDZ interaction in primary cultures of hippocampal and cortical neurons from C57BL/6J mice. Neurons overexpressing NOS1AP isoforms or deletion mutants showed highly altered spine morphology and excessive growth

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of filopodia-like protrusions. Sholl analysis of immunostained primary cultured neurons revealed that dendritic branching was mildly affected by NOS1AP overexpression. Our results hint towards an involvement of NOS-I/NOS1AP interaction in the regulation of dendritic spine plasticity. As altered dendritic spine development and filopodial outgrowth are important neuropathological features of schizophrenia, our findings may provide insight into part of the molecular mechanisms involved in brain morphology alterations observed in schizophrenia. As the NOS-I/NOS1AP interface can be targeted by small molecules, our findings ultimately might help to develop novel treatment strategies for schizophrenia patients.
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1. Introduction

Schizophrenia is a severe mental disorder that is characterized by a variety of positive (e.g., delusions, hallucinations, psychosis, paranoia), negative (e.g. blunted affect, anhedonia, lack of motivation) and cognitive symptoms (e.g., poor attention, memory deficits). This disorder occurs due to a complex interaction of genes, environment and biochemical imbalances in the brain (reviewed in Lewis and Lieberman, 2000). Current antipsychotic treatment mainly targets the dopaminergic and serotonergic systems. Although these drugs are quite effective in treating positive symptoms, negative symptoms and cognitive deficits persist (Horacek et al., 2006), suggesting that other systems are also involved in the pathophysiology of schizophrenia. Studies in animals and humans targeting the N-methyl-D-aspartate (NMDA) receptor revealed that NMDA receptor hypofunction is an important contributor to these treatment-resistant symptoms (reviewed in Coyle, 2006). NMDA receptors at the post-synaptic membrane interact with a large protein complex including membrane associated guanylate cyclases (e.g. PSD-95 and PSD-93) and Shank proteins. This macromolecular protein complex, named post-synaptic density (PSD) due to the high electron density observed in electronmicrography, enables proper clustering of proteins in the glutamatergic post-synapse (reviewed in Boeckers, 2006). Disturbed integrity of the PSD has been associated with different psychiatric conditions including schizophrenia (reviewed in De Bartolomeis et al., 2014; Hall et al., 2014; Ting et al., 2012) and postmortem studies from schizophrenia patients revealed altered gene expression and protein levels of PSD proteins in cortical brain regions (e.g., Catts et al., 2015; Kristiansen et al., 2006). In addition, reduced glutamatergic neurotransmission has also been shown in cortical and limbic areas of schizophrenia patients (Hu et al., 2015). These findings suggest a critical involvement of glutamatergic signaling in the pathophysiology of schizophrenia.

In a number of case-control and genome wide association studies one particular component of the glutamatergic post-synapse, neuronal nitric oxide (NO) synthase (NOS-I) encoded by the *NOS1* gene, has been shown to be associated with schizophrenia (Bernstein et al., 2005; Freudenberg et al., 2015; Reif et al., 2006; Weber et al., 2014). NOS-I is the major source of NO in the central nervous system and its enzymatic activity is dependent on Ca^{2+} and calmodulin, and requires dimerization of NOS-I. NOS-I carries an extended PDZ domain consisting of the core PDZ domain followed by the so-called β-finger, which contains an internal PDZ motif (-ETTF-; see Fig. 1a) which interacts with the PDZ2 domain of PSD-95 (and also PSD-93), anchoring NOS-I to the PSD (Tochio et al., 1999; Wang et al., 2000). The PDZ1 or PDZ2 domain of PSD-95 in turn bind to the GluN2 subunits of NMDA receptors, bringing NOS-I in proximity to NMDA receptors, allowing NMDA receptor mediated Ca^{2+} influx to activate NOS-I. Therefore, the NOS-I/PSD-95/NMDA receptor complex appears to be critical for the physiological integrity of NOS-I signaling at the glutamatergic post-synapse (Freudenberg et al., 2015). The PDZ domain of NOS-I interacts, among others, with an internal ExF motif (-ECF-) (Li et al., 2015) and the carboxyterminal PDZ-motif (-IAV) (Jaffrey et al., 1998) of NOS-I adapter protein (NOS1AP, previously named CAPON; Fig. 1a). NOS1AP binding to NOS-I directly competes with the interaction between NOS-I and PSD-95 and alters subcellular localization of NOS-I (Jaffrey et al., 1998). NOS1AP is also an attractive positional and functional candidate gene according to the findings of previous linkage, association and functional studies (Brzustowicz, 2008). Moreover, increased expression of NOS1AP was shown in postmortem brains of patients with schizophrenia (Hadzimichalis et al., 2010; Xu et al., 2005).

Disrupted maintenance of the dendritic tree and spinogenesis are two important neurophysiological features of schizophrenia (Kulkarni and Firestein, 2012; Moyer et al., 2015). Dendritic branching, which is crucial for proper signal integration in neurons (Jan and Jan, 2010), is mediated by scaffolding proteins at the PSD interfering with microtubule polarity leading to reorganization of the neuronal cytoskeleton forming dendritic protrusions called filopodia which may be stabilized to dendritic branches (Charych et al., 2006; Sweet et al., 2011). Dendritic filopodia (up to 20 μm in length) are also suggested to be replaced by shaft synapses and dendritic spines in developing neurons, indicating a potential role for filopodia as dendritic spine (up to 2–3 μm in length) precursors (Sweet et al., 2011; Yuste and Bonhoeffer, 2004). Reduced mature spine density (e.g., stubby, thin and mushroom) and increased filopodia-like structures in mature neurons have been shown to be related to impaired cognitive functions (De Bartolomeis et al., 2014; Penzes et al., 2011) as commonly observed in patients with schizophrenia.

Overexpression of NOS1AP has been shown to regulate dendritic spine development and dendrite patterning by reducing the number of dendritic protrusions (Carrel et al., 2009; Richier et al., 2010; Zhu et al., 2014). However, we still do not have a clear understanding how different NOS1AP domains specifically contribute to dendritic plasticity. Here, we have constructed viral vectors expressing

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