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Research Report

Alpha-synuclein is associated with the synaptic vesicle apparatus in the human and rat enteric nervous system

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ABSTRACT

Background and aims: Aggregation of alpha-synuclein (a-syn) has been implicated in the development of neurodegenerative diseases including its spread from the enteric nervous system (ENS) to the brain. Physiologically, a-syn is located at the presynapse and might be involved in regulating of neurotransmission. Therefore, the aim of the study was to characterize the physiological ontogenetic and locoregional expression pattern of a-syn in the ENS and its association with the synaptic vesicle apparatus.

Material and methods: Ontogenetic mRNA expression of a-syn and synaptophysin was determined in the rat intestine. Myenteric plexus cultures treated with glial cell line-derived neurotrophic factor (GDNF) were assessed for mRNA expression of a-syn, co-localization of a-syn with the pan-neuronal marker PGP 9.5 and the synaptic vesicle marker synaptophysin and studied by scanning electron microscopy (SEM). Human colonic specimens were subjected to co-localization studies of a-syn with synaptophysin.

Results: a-syn and synaptophysin intestinal gene expression levels were highest during early postnatal life and also detectable at adult age. a-syn was co-localized with PGP 9.5 and synaptophysin in myenteric plexus cultures and up-regulated after GDNF treatment. SEM confirmed the presence of neuronal varicosities to which a-syn was associated. Consistently, a-syn and synaptophysin showed partial co-localization in the human ENS.

Abbreviations: a-syn, alpha synuclein; GI, gastrointestinal; ENS, enteric nervous system; PD, Parkinson's disease; GDNF, glial cell line-derived neurotrophic factor; TGF-ß, transforming growth factor-ß; SMP, submucosal plexus; MP, myenteric plexus; P, postnatal day

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Conclusions: The ontogenetic and cellular expression pattern as well as the regulation by GNDF give evidence that a-syn is physiologically associated to the synaptic vesicle apparatus. The data suggest that a-syn is involved in the regulation of synaptic plasticity in the ENS during early postnatal life and adult age.

1. Introduction

Alpha-synuclein (a-syn) is a 140 amino acids comprising protein and a member of the synuclein family (Clayton and George, 1998). Under pathological conditions, aggregated a-syn as a component of Lewy bodies has been implicated in several neurodegenerative diseases collectively described as a-synuclein aggregation diseases. Consequently, a role for aggregated a-syn in the progression of these diseases has been proposed for Parkinson's disease (PD), PD dementia, and dementia with Lewy bodies (Baba et al., 1998; Spillantini et al., 1998; Ueda et al., 1993). However, the pathophysiology of the neurodegenerative process can hardly be explained by the presence and frequency of Lewy bodies (Schulz-Schaeffer, 2010). The vast majority of a-syn aggregates were recently found to be located at presynaptic terminals as faint deposits, indicating a degenerative process at the presynapse.

In contrast to the well documented evidence for the involvement of a-syn aggregations in neurodegenerative diseases, the physiological functions of this protein remain largely elusive. Under non-pathological conditions a-syn can be detected in various subcellular compartments with specific enrichment in presynaptic terminals (Lavedan, 1998; Yu et al., 2007). Thus, it has been hypothesized that a-syn is involved in the regulation of synaptic plasticity (Cheng et al., 2011). a-syn promotes the SNARE complex assembly and maintains the size of the pre-synaptic vesicular pool and the vesicle recycling (Abeliovich et al., 2000; Bonini and Giasson, 2005; Larsen et al., 2006; Yavich et al., 2004).

We recently demonstrated that a-syn is abundantly expressed in the human enteric nervous system (ENS) of individuals unaffected by synuclein aggregation diseases (Böttner et al., 2012) raising the question of the physiological role of a-syn in the gut. The ENS is considered as “brain-in-the-gut” or “enteric minibrain” and contains more than 150 million nerve cells constituting an integrative neuronal network composed of intramural ganglia and interconnecting nerve fibers arranged in two major nerve plexuses, the submucosal plexus (SMP) and myenteric plexus (MP). Survival, differentiation and maintenance of enteric neurons are strongly influenced by neurotrophic factors. Glial cell line-derived neurotrophic factor (GDNF) is a key neurotrophin for the ENS and a member of the TGF-ß superfamily of growth factors which regulate numerous functions in the development and differentiation of the nervous system (Böttner et al., 2000). The impact of the GDNF system on the ENS became evident, when gene-ablated animal models were analyzed for ENS defects as deletion of GDNF leads to total intestinal aganglionosis, i.e. the complete loss of enteric neurons in the small and large intestine (Moore et al., 1996).

Given the involvement of a-syn in synaptic plasticity and the association of a-syn with the synaptic vesicle apparatus in the central nervous system, we aimed to characterize comparable roles of native a-syn at the level of the ENS. To monitor the expression profile of a-syn during ENS maturation characterized by synaptogenesis we performed an ontogenetic study in the rat intestine with parallel assessment of the expression pattern of the synaptic vesicle marker synaptophysin. Furthermore, we used myenteric plexus cultures stimulated by GDNF as an in vitro model of the developing ENS and monitored the expression profile and cellular distribution pattern of a-syn. Finally, we aimed to transfer the results derived from the animal and cell culture models to the human ENS to provide a basis for a better understanding of the role of a-syn in the pathogenesis of neurodegenerative diseases involving the ENS.

2. Results

2.1. *Ontogenetic mRNA expression of a-syn and synaptophysin in the intestine of rats*

To monitor the ontogenetic mRNA expression profiles of a-syn and synaptophysin in the intestine, rats of P0, P3, P6, and P21 as well as adult animals were examined. mRNA levels of a-syn in the small intestine decreased postnatally as rats of P3, P21 and adult age showed significantly lower a-syn mRNA contents compared to P0 rats (Fig. 1A). A similar expression profile was observed for synaptophysin in the small intestine; all ages investigated demonstrated lower synaptophysin mRNA levels compared to P0 rats (Fig. 1B). In the colon a-syn mRNA expression showed a tendency to decrease at P21 and in adult rats compared to P0 animals, however without statistical significance (Fig. 1C). Synaptophysin mRNA expression, however, significantly decreased in the colon of P21 and adult rats compared to P0 rats (Fig. 1D). Taken together, a-syn and synaptophysin showed strikingly similar gene expression profiles in the small and large intestine during rat ontogenesis.

2.2. *Effects of GDNF on a-syn mRNA expression in myenteric plexus cultures*

As we aimed to investigate the effect of the neurotrophic factor GDNF on a-syn mRNA expression, we implemented a cell culture model of rat postnatal dissociated myenteric nerve cells exposed to increasing concentrations of GDNF. After one week of GDNF treatment, a-syn expression was 3.7 fold up-regulated in cultures treated with 50 ng/ml GDNF compared to controls (Fig. 2).