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Combinational losses of synucleins reveal their differential requirements for compensating age-dependent alterations in motor behavior and dopamine metabolism

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ABSTRACT

Synucleins are involved in multiple steps of the neurotransmitter turnover, but the largely normal synaptic function in young adult animals completely lacking synucleins suggests their roles are dispensable for execution of these processes. Instead, they may be utilized for boosting the efficiency of certain molecular mechanisms in presynaptic terminals, with a deficiency of synuclein proteins sensitizing to or exacerbating synaptic malfunction caused by accumulation of mild alterations, which are commonly associated with aging. Although functional redundancy within the family has been reported, it is unclear whether the remaining synucleins can fully compensate for the deficiency of a lost family member or whether some functions are specific for a particular member. We assessed several structural and functional characteristics of the nigrostriatal system of mice lacking members of the synuclein family in every possible combination and demonstrated that stabilization of the striatal dopamine level depends on the presence of α-synuclein and cannot be compensated by other family members, whereas β-synuclein is required for efficient maintenance of animal's balance and coordination in old age.

1. Introduction

Among several proteins whose malfunction has been linked to molecular events leading to neuronal dysfunction in Parkinson's disease (PD), α-synuclein deservingly hold the status of a primary culprit due to its causative role in certain familial forms and a pivotal role in the formation of histopathological hallmarks in both familial and idiopathic forms of the disease [reviewed in (Dev et al., 2003; Venda et al., 2010)]. Moreover, polymorphisms within the locus encoding α-synuclein have been found to be associated with the increased risk of PD development (Kay et al., 2008; Mizuta et al., 2008; Pankratz et al., 2009; Scholz et al., 2009; Sutherland et al., 2009), with recent studies suggesting that disease progression to the symptomatic stage correlates with the spreading of pathologic α-synuclein aggregates to dopaminergic neurons of the substantia nigra pars compacta (SNpc) (Braak et al., 2006; Desplats et al., 2009; Luk et al., 2012; Recasens et al., 2014).

Aggregation of α-synuclein not only generates various products intrinsically toxic for neurons but also depletes the pool of functional α-synuclein, particularly in presynaptic terminals, its normal site of localization and function. The importance of α-synuclein for the efficient function of vertebrate presynaptic neurotransmitter storage and release machinery has been demonstrated in experiments with live animals or primary neurons derived from either animals overexpressing various forms α-synuclein or depleted of endogenous α-synuclein by targeted inactivation of *Snca*, gene encoding mouse α-synuclein (Abeliovich et al., 2000; Cabin et al., 2002; Chandra et al., 2004, 2005; Janezic et al., 2013; Lim et al., 2010; Lin et al., 2012; Nemani et al., 2010; Senior et al., 2008; Taylor et al., 2014; Yavich et al., 2004, 2005, 2006).

However, in models with transgenic α-synuclein expression, it is problematic to identify the chain of events leading to alterations of synaptic functions. A modified or overexpressed exogenous α-synuclein may hijack and compromise the endogenous α-synuclein

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function. Alternatively, functional alterations may be caused by the novel gain-of-function effects of the exogenous α-synuclein, which can be completely unrelated to any of normal functions of the endogenous protein or affect them only indirectly. On the other hand, interpretation of experimental data obtained in α-synuclein null mutant mice is complicated by the expression in vertebrate neurons, including dopaminergic neurons of SNpc, of 2 closely related members of the synuclein family, β-synuclein and γ-synuclein. These 2 proteins are not known to be directly involved in etiology or pathogenesis of PD but can function in the same molecular processes in neuronal synapses as α-synuclein and therefore, compensate, at least partially, for its loss. This functional redundancy might mask consequences of α-synuclein depletion in the SNpc of null mutant mice.

In the absence of the interfamily compensation, that is, following inactivation of all 3 synuclein-encoding genes, animals develop more pronounced behavioral, physiological, and biochemical changes in their nervous system, including the nigrostriatal axis, than animals with null mutations for 1 or 2 of these genes. Importantly, synaptic malfunction develops gradually and its manifestations become detectable only in aging triple synuclein null mutant mice (Anwar et al., 2011; Burre et al., 2010; Greten-Harrison et al., 2010). These observations suggest that the presence of synucleins delays functional decline of neuronal synapses in the aging nervous system. However, the impact of each family member on this process might be different. Indeed, a significant, although not sufficient to cause functional alterations, decrease of striatal dopamine level has been observed only in adult α/β-synuclein double and aged α-synuclein but not in adult α/γ-synuclein double and aged γ-synuclein null mutant mice (Al-Wandi et al., 2010; Chandra et al., 2004; Robertson et al., 2004).

For synucleins, as any other proteins that affect efficiency rather than execution or robust regulation of intracellular processes, it is particularly important to consider effects of external factors when comparing data obtained in different setups. In particular, for studies of mice lacking such proteins, genetic background and environmental conditions might significantly affect experimental data, and at least some discrepancies in results obtained in similar studies of synuclein null mutant mice carried out in different laboratories may have been caused by differences in these parameters.

Therefore, to address the role of each member of the synuclein family in maintaining the efficiency of the nigrostriatal system function in aged animals, we carried out a breeding programme aimed to produce mouse lines lacking 1, 2, or all 3 synucleins and control wild-type mice, on the same C57Bl6/J genetic background, and systematically studied motor behavior and major parameters of the nigrostriatal system in cohorts of aged mice of all 8 genotypes.

2. Material and methods

2.1. Generation of double and triple synuclein null mutant animals

Generation of α-synuclein, γ-synuclein, and α-synuclein/γ-synuclein double null mutant mice on C57Bl6J (Charles River) background was described previously (Abeliovich et al., 2000; Ninkina et al., 2003; Robertson et al., 2004). Heterozygous β-synuclein null mutant mice (Chandra et al., 2004) on C57Bl6J background were further backcrossed with C57Bl6J (Charles River) mice for 6 generations in the Cardiff University Transgenic Animal Unit before breeding with α-synuclein/γ-synuclein double null mutant mice. Resultant triple heterozygous animals were intercrossed to produce founders of triple null mutant, double null mutant, and wild-type colonies used in this study. Thus, all studied animals were on the same C57Bl6J (Charles River) genetic background. Mice were maintained in conventional open-lid cages with ad libitum access to standard chow and water. Mouse genotyping was carried out as described previously (Chandra et al., 2004; Ninkina et al., 2003; Robertson et al., 2004). Animals were sacrificed by a Schedule 1 method or terminally anesthetized followed by perfusion fixation, and tissues were collected and coded. Individuals that performed all further analyses were blinded to the sample genotype. All animal work was carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986).

2.2. Behavioral tests

2.2.1. Inverted grid test

Mice were placed onto a 30 cm by 30 cm square mesh consisting of 5-mm squares of 0.5-mm diameter wire. The grid was slowly rotated to the inverted position and held above a thick layer of bedding material. If a mouse fell from the grid earlier than the maximum test time of 1 minute the latency to fall was noted, and after a 10-minute rest period in the home cage, the test was repeated. The best result from 3 attempts was included in the statistics.

2.2.2. Rotarod test

Mice were trained and then tested for 5 minutes on the accelerating (4–40 rpm) Ugo Basile 7650 rotarod as described previously (Robertson et al., 2004). Each mouse was tested 3 times with at least 30-minute rest period between trials. The mean latency to fall for these 3 trials was included in the final statistics.

2.3. Immunohistochemistry and neuronal cell counts

Mouse brains were collected, fixed with Carnoy's fixative, processed, embedded, and cut using an HM 310 microtome (Microm International). Sections of 8 μm thick were mounted onto poly-L-lysine–coated slides followed by staining with antibodies against tyrosine hydroxylase ([TH], mouse monoclonal antibody, clone TH-2, Sigma) diluted 1:1000. For detection, secondary biotinylated anti-mouse or anti-rabbit antibodies, Elite plus kits (Vector laboratories), and 3,3'-diaminobenzidine as chromogen for the peroxidase reaction were used. The borders of the substantia nigra and ventral tegmental area (VTA) on these sections were outlined using distribution atlas of TH-positive cells (Hokfelt et al., 1984). The number of TH-positive neurons was assessed by stereological counting as described in our previous publications (Al-Wandi et al., 2010; Robertson et al., 2004). Briefly, the first section for counting was randomly chosen from the first 10 sections that included SNpc/VTA region. Starting from this section, every TH-positive cell with a clearly seen nucleus was counted on the every fifth section through the whole region. The Axiovision imaging program (Carl Zeiss Vision) was employed to measure diameters of 30 nuclei of dopaminergic neurons in the SNpc of every mouse brain included in this study. The nuclei were chosen randomly and the distance measured as the horizontal length as they appeared on screen. A mean was calculated for each animal and used for Abercrombie's correction (Abercrombie, 1946) to obtain an actual number of TH positive cells in the structure.

2.4. High-pressure liquid chromography (HPLC) analysis of striatal neurochemicals

Mouse dorsal striata were dissected, immediately snap-frozen in liquid nitrogen, and kept at −80 °C. After extraction with 0.06-M $HClO_4$, concentrations of striatal dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were measured by HPLC with electrochemical detection using a 4.6 × 150-mm Microsorb C18