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Research Paper

Functional alterations of the dopaminergic and glutamatergic systems in spontaneous α-synuclein overexpressing rats

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ABSTRACT

The presence of α-synuclein (α-syn) in Lewy bodies and Lewy neurites is an important characteristic of the neurodegenerative processes of substantia nigra pars compacta (SNpc) dopaminergic (DAergic) neurons in Parkinson's disease (PD) and other synucleinopathies.

Here we report that Berlin-Druckrey rats carrying a spontaneous mutation in the 3′ untranslated region of α-syn mRNA (*m/m* rats) display a marked accumulation of α-syn in the mesencephalic area, striatum and frontal cortex, accompanied to severe dysfunctions in the dorsolateral striatum. Despite a small reduction in the number of SNpc and ventral tegmental area DAergic cells, the surviving dopaminergic neurons of the *m/m* rats do not show clear-cut alterations of the spontaneous and evoked firing activity, DA responses and somatic amphetamine-induced firing inhibition. Interestingly, mutant DAergic neurons display diminished whole-cell *I*h conductance and a reduced frequency of spontaneous excitatory synaptic currents. By contrast, *m/m* rats show a severe impairment of DA and glutamate release in the dorsolateral striatum, as revealed by amperometric measure of DA currents and by electrophysiological recordings of glutamatergic synaptic events in striatal medium spiny neurons. These functional impairments are paralleled by a decreased expression of the DA transporter and VGluT1 proteins in the same area. Thus, together with α-syn overload in the mesencephalic region, striatum and frontal cortex, the main functional alterations occur in the DAergic and glutamatergic terminals in the dorsal striatum of the *m/m* rats.

1. Introduction

Pathological misfolding and aggregation of alpha-synuclein (α-syn) has been genetically linked to familial forms of PD (Polymeropoulos et al., 1997; Krüger et al., 1998; Spillantini et al., 1998; Zarranz et al., 2004), as well as, other related neurodegenerative diseases, which are collectively known as `synucleinopathies'. This term besides PD, also includes dementia with Lewy bodies and multisystem atrophy (Surguchov, 2008; Barker and Williams-Gray, 2016). The latter is mainly characterized by α-syn aggregates in oligodendroglia (Jellinger, 2014). In normal conditions, α-syn is abundant in synaptic terminals where it could modulate vesicle trafficking and neurotransmitter release (Maroteaux et al., 1988; Bellucci et al., 2012; Anwar et al., 2011; Senior et al., 2008;Yavich et al., 2005). On the other hand, misfolded α-syn may exert toxic functions (Norris et al., 2004; Cookson and Van der Brug, 2008; Bellucci et al., 2015) or cause loss of defensive properties

Abbreviations: α-syn, α-synuclein; *m/m*, homozygous mutant rats; +/+, control animals; PD, Parkinson's disease; LBs, Lewy bodies; SNpc, substantia nigra pars compacta; VTA, ventral tegmental area; DA, dopamine; DAergic neurons, dopaminergic neurons; MSNs, medium spiny neurons; MEA, multi-electrode array; CPA, constant potential amperometry; DAT, dopamine transporter; HCN, hyperpolarization-activated cyclic nucleotide-gated channels; GIRK, G-protein coupled inward rectifier potassium channels; MPH, methylphenidate; PPR, paired-pulse ratio; TH, tyrosine hydroxylase.

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(da Costa et al., 2000). Accordingly, recent evidence indicates that oligomeric α-syn, forming fibrils found also extracellularly (Martin et al., 2012), is the toxic species that could start the neuropathological processes (Cookson and van der Brug, 2008; da Costa et al., 2000).

Animal models aimed to determine the physiological and pathological role of this protein in the DAergic nigrostriatal neurons have recently been developed but, to date, there is no clear information on the functional alterations caused by α-syn accumulation at somatic, dendritic and terminal level of SNpc DAergic cells. α-syn participates to the synaptic physiology of the DAergic cells (Bellucci et al., 2012; Bellucci et al., 2015), thus, mice lacking α-syn display an alteration in the modalities of DA release, being decreased the refilling of the vesicular content of this catecholamine in readily releasable pools (Chadchankar et al., 2012; Abeliovich et al., 2000).

On the other hand, α-syn over-expression determines less excitatory synaptic vesicle exocytosis in cultured DAergic neurons, which release glutamate beside DA (Nemani et al., 2010). In addition, insertion of a truncated form and increased expression of α-syn in the nigrostriatal system of transgenic mice diminishes vesicle density and reduces DA release (Garcia-Reitböck et al., 2010). This results in a certain degree of motor impairment (Tofaris et al., 2006; Gaugler et al., 2012).

Concerning cellular integrity, it has been reported that an increased production of α-syn by viral infection causes DAergic cell loss (Lundblad et al., 2012; Gaugler et al., 2012). Of note, bacterial artificial chromosome (BAC) transgenic rats expressing the human full-length wild-type α-syn, develop intracellular α-syn-containing insoluble precipitates which cause alteration of the DAergic system. Thus, these animals show early changes in novelty-seeking, avoidance and smell, followed, at later stages, by progressive motor deficits closely resembling human PD (Nuber et al., 2013).

Recently, a spontaneous autosomal recessive rat model has been discovered, characterized by multisystemic neurodegeneration that rapidly recapitulates some pathological features of PD and possibly, dementia with Lewy bodies (Stoica et al., 2012). During the first weeks of age, these rats display an increase in α-syn in the whole brain, modifications of dendritic spines in striatal medium spiny neurons and a severe loss of tyrosine-hydroxylase positive terminals in the striatum. These pathological aspects could be linked to the presence of a point mutation in the 3′ untranslated region (UTR) of the α-syn mRNA that may increase or deregulate transcription of the protein (Stoica et al., 2012).

In the attempt to assess if the morphological and biochemical modifications occurring in this rat model of neurodegenerative disease are linked to functional alterations in the basal ganglia circuitry and, particularly, of the nigrostriatal DAergic and cortico-striatal glutamatergic systems, here we performed in vitro electrophysiological and amperometric recordings plus stereological cell count of TH-stained neurons in the midbrain. Additionally, we quantified the expression levels of dopamine and vesicular glutamate transporters 1 and 2 in striatal synaptic terminals. We found that at advanced stages of the disease besides abnormal α-syn accumulation in the mesencephalic area, striatum and frontal cortex, there is a severe functional alteration of the DAergic and glutamatergic neurotransmission within the dorsal striatum, whereas only minor abnormalities are found in the mesencephalic area.

2. Materials and methods

2.1. Animals

All experiments were carried out in accordance with the international guidelines on the ethical use of animals from the EU Directive 2010/63/EU for animal experiments and the Ethics Committee of the University of Tor Vergata, Rome, Italy. The spontaneously inherited autosomal recessive rat model for neurodegeneration was provided by Prof. Stoica, Texas AM University (Stoica et al., 2012). Affected offspring (*m*/*m*) were identified by the gray color of their head coat and by the appearance of motor symptoms at P15 which became widespread at P27–30. For ethical consideration *m*/*m* animals were sacrificed not later than P30. Control animals (black head coat, +/+) were identified by two consecutive breedings with heterozygous partners, in which they did not generate *m*/*m* offspring.

2.2. Slice preparation for electrophysiology

Preparation of midbrain slices was performed as described previously (Lacey et al., 1989; Mercuri et al., 1995) from control or *m*/*m* rats (P15–P30, either sex). Horizontal slices (thickness 250 or 300 μm) containing the substantia nigra pars compacta (SNpc) and the ventral tegmental area (VTA), were cut with in ice-cold modified artificial CSF (ACSF). This solution contained (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 10 glucose, and 24 $NaHCO_3$, 290 mOs/L, and was gassed with 95% O_2–5% CO_2, pH 7.4. Coronal cortico-striatal slices (thickness 250 μm) were prepared accordingly to Geracitano et al. (2003) from P27 control or *m*/*m* rats and were cut in ACSF at room temperature.

2.3. Whole cell patch clamp recordings in SNpc DAergic and medium spiny neurons

Recordings in SNpc dopaminergic (DAergic) neurons were performed accordingly to Guatteo et al. (2013). SNpc DAergic neurons, localized in a region of tightly packed neurons adjacent to the medial terminal nucleus of the accessory optic tract were identified based on a slow and regular spontaneous firing (1–5 Hz), hyperpolarizing/outward response to dopamine (DA, 30 μM), prominent *I*h in response to hyperpolarizing voltage steps (−60 to −120 mV, 20 mV increment, holding potential, V_h, −60 mV, Fig. 2 A inset; Mercuri et al., 1995) and, in current-clamp mode, a depolarizing sag upon application of negative currents (Guatteo et al., 2000). Patch pipettes (2–5 MΩ) were filled with a solution containing (in mM): K-gluconate (135), $CaCl_2$ (0.1), $MgCl_2$ (2), KCl (10), EGTA (0.75), HEPES (10), Mg2-ATP (2), and Na3-GTP (0.3), pH 7.3, osmolarity 280 mOs/L. Ih amplitude was measured as difference between instantaneous and steady-state current at −120 mV (Fig. 2 A insets, dotted lines and arrows). In a subset of experiments, we utilized a different protocol to study *I*h voltage dependence and whole cell conductance (*G*h) in DAergic neurons, consisting of 2 s conditioning step at different membrane potentials (−70 to −130 mV, 10 mV increment, V_h – 60 mV, Fig. 2 E, inset) followed by 300 ms test potential at −140 mV to elicit tail currents. Tail currents were normalized (I/Imax) for their maximal amplitude obtained at −130 mV pre-conditioning pulse.

Medium spiny neurons (MSNs) were identified in dorsolateral striatum slices based on their negative membrane potential (<−80 mV) and the presence of firing in response to depolarizing current steps (+200 pA). Pipette solution for current clamp recordings had the following composition (in mM): K-gluconate (120), KCl (20), MgCl2 (2), EGTA (0.2), HEPES (10), Mg-ATP (4), Na-GTP (0.3), osmolarity 275–285 mOs/L, pH 7.2. Excitatory post-synaptic currents (EPSCs) were evoked in MSNs (V_h – 70 mV) by a bipolar Ni/Cr insulated stimulating electrode placed in the corpus callosum to activate cortico-striatal fibers, in the presence of picrotoxin (100 μM) to block GABA-A mediated currents. Recordings of sEPSCs were made with a pipette solution containing (in mM): Cs-methanesulfonate (120), CsCl (15), NaCl (8), HEPES (10), EGTA (0.2), TEA-Cl (10), QX314-Cl (5), Mg-ATP (2), Na-GTP (0.3), osmolarity 275–285 mOs/L, pH 7.2.

Paired pulse ratio (PPR) was calculated from two consecutive EPSCs at V_h of −70 (100 ms interval, EPSC2/EPSC1). To measure AMPA/NMDA ratio, EPSCs were evoked at V_h of −70 and +40 mV respectively. Maximal NMDA current amplitude was measured at 60 ms delay after the stimulus artifact (Fig. 9 D, arrows), when AMPA current was completely inactivated.