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Ventral tegmental area dopamine neurons are resistant to human mutant alpha-synuclein overexpression

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Parkinson's disease (PD) is characterized by the formation of intracytoplasmic inclusions, which contain α-synuclein (α-syn) protein. While most profound neurodegeneration is seen in the dopamine (DA) synthesizing neurons located in the ventral midbrain, it is unclear why some DA cell groups are more susceptible than others. In the midbrain, the degeneration of the substantia nigra (SN) DA neurons is severe, whereas the involvement of the ventral tegmental area (VTA) neurons is relatively spared. In the present study, we overexpressed human A53T α-syn in the VTA neurons and found that A53T toxicity did not affect their survival. There was, however, a mild functional impairment seen as altered open field locomotor activity. Overexpression of A53T in the SN, on the other hand, led to profound cell loss. These results suggest that the selective susceptibility of nigral DA neurons is at least in part associated with factor(s) involved in handling of α-syn that is not shared by the VTA neurons. Secondly, these results highlight the fact that impaired but surviving neurons can have a substantial impact on DA-dependent behavior and should therefore be considered as a critical part of animal models where novel therapeutic interventions are tested.

Keywords: Parkinson's disease; Recombinant adeno-associated virus; α-synuclein; Mesolimbic; Cell death; Stereology; Tyrosine hydroxylase; Open field activity

Introduction

In Parkinson's disease (PD), the aggregation of proteins in the cytoplasm of cells leads to formation of inclusions, termed Lewy bodies (Lewy, 1912). While many neuronal cell groups are affected in PD (Braak et al., 2004), it is predominantly a dopamine (DA) deficiency syndrome and is characterized by the loss of dopaminergic neurons in the ventral midbrain (Bernheimer et al., 1973; Kish et al., 1988; Fearnley and Lees, 1991). The vulnerability of DA neurons in PD is clear; however, it is not known why these cells are more susceptible to neurodegeneration than other systems in the brain. Furthermore, different cell groups within the dopaminergic system display different susceptibilities to cell death. Degeneration of the DA neurons in the substantia nigra (SN) is the most prominent, whereas, in the ventral tegmental area (VTA), DA neurons are less affected (Uhl et al., 1985; Hirsch et al., 1988; Kish et al., 1988; Damier et al., 1999; Braak et al., 2004). This phenomenon is also evident in animal models following the administration of toxic substances such as 6-OHDA in rats, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice and primates rotenone, and proteasome inhibitors (Grant and Clarke, 2002; German et al., 1988, 1996; Lavoie and Parent, 1991; Betarbet et al., 2000; McNaught et al., 2004). Furthermore, differences in cell susceptibility are evident in Zitter rats, which display an abnormal metabolism of H_2O_2 (Gomi et al., 1994; Grant and Clarke, 2002). Data from these studies, many of which are related to oxidative stress, clearly show that there are differences between the SN and VTA in their susceptibility to cell death induced by toxic agents.

Appearance of a familial PD phenotype in an autosomal dominant manner has been described to result from point mutations in the α-syn gene (Polymeropoulos et al., 1997; Kruger et al., 1998; Zarranz et al., 2004). In addition, recent reports showed that familial PD also occurs in cases where multiplications of the α-syn gene have been identified, leading to an overexpression of wild-type α-syn protein (Farrer et al., 2001; Singleton et al., 2003; Chartier-Harlin et al., 2004). These findings stimulated several investigators to study the function of the α-syn protein. Today, a variety of functions including synaptic modulation, neurotransmitter regulation, synaptic maintenance, neuronal regeneration and chaperone activity have been demonstrated (Murphy et al., 2000; Lee et al., 2001; Masliah et al., 2000; Perez et al., 2002; Cabin et al., 2002; Abeliovich et al., 2000; Jenco et al., 1998; Duda et al., 1999; Quilty et al., 2003; Souza et al., 2000; Ostrerova et al., 1999; Park et al., 2002).

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In parallel, new animal models have been developed using recombinant viral vectors to overexpress human α-syn protein. Recombinant adeno-associated virus (rAAV) and lentiviral vectors were used to overexpress either wild-type or the mutated forms of human α-syn in the SN of rats and primates (Kirik et al., 2002, 2003; Klein et al., 2002; Lo Bianco et al., 2002, 2004; Yamada et al., 2004, 2005). In the rat model, the production of human wild-type or mutated α-syn protein is detectable at the level of cell bodies already at 1-week post-injection. At 2–3 weeks after transduction, human α-syn fills up the processes of the nigral neurons extending to the striatum. Finally, increased α-syn load leads to a progressive neurodegeneration in the SN with an onset between 3 and 5 weeks. During this period, large α-syn-positive inclusions are seen throughout the dopaminergic cells, where some cell bodies are found to be shrunken, as well as dystrophic dendrites and axonal projections are prominent. The magnitude of nigral cell loss, as measured by the number of surviving TH-positive cells at 8 weeks in rAAV2-α-syn-injected animals varies between 30% and 80% (Kirik et al., 2002). However, in these previous experiments, the overexpression of the transgenic α-syn was targeted to the SN, therefore vulnerability of the VTA neurons could not be assessed.

In the present experiment, we tested if the DA neurons located in the VTA were resistant to the overexpression of human A53T mutated α-syn.

Materials and methods

Animals

A total of 68 adult female Sprague–Dawley rats were used in this study (B&K Universal AB, Stockholm, Sweden). The animals were housed three to a cage under a 12 h light/dark cycle with ad libitum access to food and water in the facilities of the Biomedical Center at Lund University. All procedures described here were approved by the Ethical Committee for the use of laboratory animals at Lund/Malmö region.

Production of recombinant viral vectors

The rAAV2-A53T-α-syn vector contains the coding sequence for the human α-syn gene under the control of the synthetic CBA promoter (Xu et al., 2001; Kirik et al., 2002, 2003) and the human bovine growth hormone poly A site, flanked by AAV2 terminal repeats. The rAAV2-GFP vector contains the coding sequence for humanized green fluorescent protein (GFP) (Zolotukhin et al., 1996) under the control of the synthetic CBA promoter and the SV40 polyadenylation signal followed by the neomycin resistance gene under the control of the mutant polyoma virus enhancer/promoter (PYF441) and the human bovine growth hormone poly A site, flanked by AAV2 terminal repeats. rAAV2 vectors were packaged in AAV2 capsids, purified and tittered as previously described (Zolotukhin et al., 1999). The final titer for vectors encoding A53T-α-syn and GFP genes was 4.9×10^{11} and 1.5×10^{11} genome copies/ml, respectively, as determined by dot blot.

Surgical procedures and experimental groups

All surgeries were conducted under 2% isoflourane anesthesia using O_2 and N_2O gas mixture. The animals were placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA) where a 5 μl Hamilton syringe fitted with a glass capillary (outer diameter of 60–80 μm) was used as a delivery system. All animals received a single 2 μl injection of either rAAV2-GFP ($n = 14$) (GFP group) or rAAV2-A53T-α-syn ($n = 24$) vector (A53T group). A third group of lesion control animals ($n = 13$) received an injection of 9 μg of 6-OHDA (Sigma-Aldrich AB, Sweden) in 3 μl of 0.2 mg/ml ascorbic acid in saline, calculated as free-base (6-OHDA group). All injections were made into the midline of the VTA using the following anteroposterior (AP), mediolateral (ML) and dorsoventral (DV) coordinates: AP = −5.5 mm; ML = −1.6 mm; DV = −7.3 mm relative to dura; at an angle of + 13° from the vertical axis, with toothbar (TB) set at −2.3 mm. The injection coordinates and the choice of a single midline delivery site have been made based on pilot experiments where comparisons between this and a two-site lateralized injection protocol were evaluated for their reliability and reproducibility. Solutions were injected at a rate of 0.1 μl/15 s and once the injection was completed, the needle was left in position for a further 5 min before being slowly retracted. Animals were then sutured with metal clips and returned to their cage. A fourth group of normal controls ($n = 7$) (normal group) were kept naive of all surgical procedures. Finally, two further control groups were injected into the SN with 2 μl of either rAAV2-A53T-α-syn ($n = 5$) (A53T SN) or rAAV2-GFP ($n = 5$) (GFP SN) using the following coordinates: AP = −5.2 mm; ML = −2.0 mm; DV = −7.2 mm relative to dura; TB = −2.3 mm.

Behavioral tests

At 2, 6 and 10 weeks post-surgery, all animals (except those in the SN targeted groups) were subjected to activity tests in open-field chambers, each equipped with a 16 × 16 infrared photobeam system (dimensions 40.6 cm × 40.6 cm × 38.1 cm) using the Flex-Field Software system (San Diego Instruments). The testing procedures were as follows (Fig. 1): animals were habituated for 1 h during dark cycle preceding the first day of testing. The following morning, animals were placed in the chambers for 1 h to obtain baseline levels. This was followed by a subcutaneous injection of 0.1 mg/kg apomorphine in 0.02% ascorbate saline, and tested for a further 3 h. After a 48-h washout period, animals were again subjected to a baseline activity test before an intraperitoneal injection of 1.5 mg/kg amphetamine in saline and a further testing for 4 h (Fig. 1). At week 12, animals were given an i.p. injection of α-methyl-ρ-tyrosine (αMT; 60 mg/kg) and 6 h later were tested in the activity boxes for 1 h. Data from the activity tests are reported as the mean number of beam breaks per minute at baseline (calculated from the first test on day 1 and between 30 and 60 min of the test session), apomorphine-induced activity (0–60 min), amphetamine-induced activity (0–120 min) and α-MT-test (0–60 min). The data obtained following the injection of amphetamine varied largely between animals in the control groups and therefore was not further analyzed.

Histological analysis

At 14 weeks after transduction, 44 animals, including all animals from the SN-GFP and SN-A53T groups, were killed for histological analysis by an i.p. injection of sodium pentobarbital (Apoteksbolaget, Sweden) and were transcardially perfused with 100 ml saline followed by 250 ml of 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer. Brains were collected and post-fixed in 4% PFA for an additional 2 h and then moved to 25% sucrose for