The neurobiology of dysautonomia in Parkinson's disease

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A B S T R A C T

Neurodegenerative diseases (NDs) include a large variety of disorders that affects specific areas of the central nervous system, leading to psychiatric and movement pathologies. A common feature that characterizes these disorders is the neuronal formation and accumulation of misfolded protein aggregates that lead to cell death. In particular, different proteinaceous aggregates accumulate to trigger a variety of clinical manifestations: prion protein (PrP^Sc) in prion diseases, β-amyloid (Aβ) in Alzheimer's disease (AD), α-synuclein in Parkinson's disease (PD), huntingtin in Huntington's disease (HD), superoxide dismutase and TDP-43 in amyotrophic lateral sclerosis (ALS), tau in tauopathies. Non-motor alterations also occur in several viscera, in particular the gastrointestinal tract. These often precede the onset of motor symptoms by several years. For this reason, dysautonomic changes can be predictive of NDs and their correct recognition is being assuming a remarkable importance. This peculiar feature led more and more to the concept that neurodegeneration may initiate in the periphery and propagate retrogradely towards the central nervous system in a prion-like manner. In recent years, a particular attention was dedicated to the clinical assessment of autonomic disorders in patients affected by NDs. In this respect, experimental animal models have been developed to understand the neurobiology underlying these effects as well as to investigate autonomic changes in peripheral organs. This review summarizes experimental studies that have been carried out to understand autonomic symptoms in NDs, with the purpose to provide appropriate tools for comprehensive and integrated studies.

Key words

Parkinson's disease • Animal model • Dysautonomia • Gastrointestinal system • Cardiovascular system • Genitourinary system

Introduction

Neurodegenerative diseases (NDs) are chronic and progressive disorders which severely affect cognitive, memory, motor and sensory functions. Besides the appearance late in life, the marked neuronal loss, and synaptic alterations, several NDs also share the presence of neuronal deposits of altered proteins. In fact, the onset of these pathologies seems to be due to the formation and accumulation of misfolded protein aggregates which are responsible for toxic effects in neurons.

The formation and accumulation of abnormal proteins is a common feature for a group of progressive NDs. In particular, specific misfolded proteinaceous aggregates accumulate intra- and/or extracellularly to trigger a variety of NDs, accounting for different clinical manifestations: prion protein (PrP^Sc) in prion diseases, β-amyloid (Aβ) in Alzheimer's disease (AD), α-synuclein (in particular, Lewy body inclu-

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sions) in Parkinson's disease (PD), huntingtin in Huntington's disease (HD), superoxide dismutase and TDP-43 in amyotrophic lateral sclerosis (ALS), tau in tauopathies (Natale et al., 2011a,b). In some cases, proteins misfold and accumulate in multiple diseases, such as TDP-43, which was found in ALS and fronto-temporal lobar degeneration (Polymenidou and Cleveland, 2011).

Several factors may contribute to the protein misfolding and aggregation in NDs. These mainly include genetic (gene mutations of specific neuronal proteins) and environmental factors (toxic agents, oxidative damage, alterations of clearing systems such as the autophagoproteasome cascade, and abnormal protein-DNA interaction) (Jiménez, 2010; Sarkar, 2013).

Apart from motor symptoms, patients affected by several NDs also suffer from several non-motor disorders classified as autonomic, psychiatric and sensory. In particular, in PD patients, other than motor symptoms, important autonomic alterations in almost all organs and apparati also occur, with consequent worsening of the quality of life. Gastrointestinal, cardiovascular, respiratory, neuroendocrine and urogenital apparati exhibit a large variety of abnormalities. Furthermore, overall non-motor symptom progression does not seem to follow motor deterioration (Antonini et al., 2012). Interestingly, these systemic dysautonomic symptoms, mainly affecting the whole gastrointestinal tract and the cardiocirculatory system, largely anticipate the onset of the motor impairment. This led to the concept to evaluate accurately these non-motor symptoms in order to identify subjects at risk to develop PD prior to detect motor alterations (Tysnes et al., 2010; Adler, 2011). In this respect, the predictive importance of these dysautonomic disorders allowed to coin the new nosologic "Parkinson's associated risk syndrome" to describe patients that may have genetic risk factors or subtle, early non-motor symptoms including abnormalities in olfaction, gastrointestinal function, cardiac imaging, vision, behavior, and cognition (Akhtar and Stern, 2012). This task is particularly difficult when considering that enteric neurodegeneration can also occur at different extent during aging with consequent dysphagia, gastro-esophageal reflux disease, constipation, and faecal incontinence. Moreover, older subjects are also susceptible to postprandial hypotension, in which altered cardio-

vascular responses to intestinal nutrient exposure are pivotal (Camilleri et al., 2008; Wiskur and Van Meerveld, 2010; Rayner and Horowitz, 2013; Saffrey, 2013).

About 25% of autopsies from control subjects with absence of motor symptoms showed the presence of Lewy bodies, and a reduced tyrosine hydroxylase activity was also found in the striatum. This particular condition was named incidental Lewy body disease (ILBD) and can precede the onset of PD. Indeed, in many cases of ILBD the spinal cord and the orthosympathetic ganglia appear affected as in PD and the involvement of cardiovascular, genitourinary and gastrointestinal systems was described (Klos et al., 2006, Bloch et al., 2006; Jain, 2011). In these cases, the recognition of dysautonomic symptoms and signs is particularly important and may well explain what happens prior to motor PD (Wolters, 2009; Adler, 2011).

A number of data suggests that peripheral manifestations may be caused by a neurodegenerative process in the enteric nervous system (ENS) (Singaram et al, 1995; Anderson et al., 2007; Natale et al., 2010) similar to what occurs in the CNS. The mechanisms underlying protein misfolding in NDs remain controversial, while novel findings lead to hypothesize intriguing pathways responsible for altered protein transmission and propagation in a prion-like manner, in line with the Braak's hypothesis (Braak et al., 2003). In this regard, the formation and accumulation in the periphery of such prionoids would initiate the disease in the ENS and propagate retrogradely to the brain where neurologic damage would occur after several years (Angot et al., 2010; Natale et al., 2011a,b, 2013). Protein glycation and the formation of the so-called advanced glycation end-products (AGEs) would be an important step to promote aggregation and propagation of misfolded proteins in NDs, via interaction with specific receptors for AGEs (RAGEs) (Vicente-Miranda and Outeiro, 2010; Natale et al., 2011a,b).

Taking into account the importance to understand the pathophysiology of NDs, the clinical observations, the bioptic and autoptic data in humans, and the development of appropriate experimental models of disease appear essential to better characterize dysautonomic alterations in order to design effective therapeutic schedules. Since the investigation of autonomic dysfunctions in NDs is important for

determining the precise diagnosis and in predicting prognosis, the present review aims to summarize such an approach.

Observations in humans

Patients affected by a variety of NDs also exhibit autonomic disorders and several organs and apparati can be severely affected.

Synucleinopathies

The presence of misfolded α-synuclein is a characteristic of several NDs, leading to the definition of synucleinopathy. In particular, among synucleinopathies two main groups can be recognized: multiple system atrophy (MSA) with its variants (olivo-ponto-cerebellar atrophy, striato-nigral atrophy, Shy-Drager syndrome), and Lewy body diseases which include, other than PD, dementia with Lewy bodies, pure autonomic failure, Lewy body dysphagia, incidental Lewy body disease, and inherited Lewy body diseases (Goedert, 2001) (Fig. 1).

Besides resting tremor, bradykinesia, stiffness, and postural instability, in PD patients the occurrence of non-motor symptoms appears particularly relevant. Several reports described a large number of visceral alterations in PD patients. These non-motor symptoms include autonomic (gastrointestinal, cardiovascular, urinary and sexual dysfunctions; hyperhidrosis), sleep (impaired sleep initiation and maintenance, rapid eye movement disorder, excessive daytime sleepiness), sensory (pain, hyposmia, visual dysfunction), as well as neuropsychiatric (anhedonia, depression, anxiety, panic attacks, dementia, and psychosis) disorders. In particular, constipation, orthostatic dizziness, hyposmia, rapid eye movement disorder and depression are the most
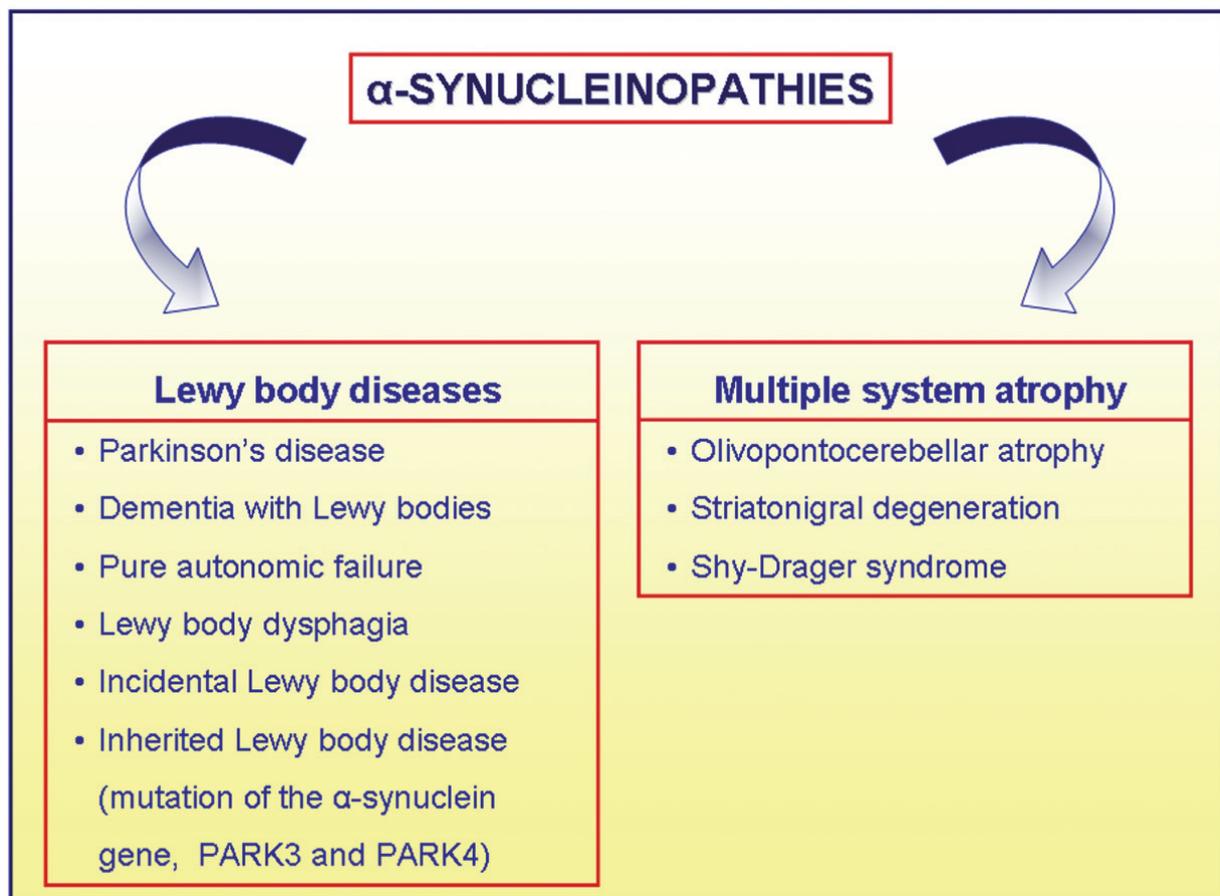

Fig. 1. - Classification of synucleinopathies. Modified from Goedert (2001).

frequent symptoms that can anticipate the motor impairment (Chaudhuri et al., 2005, 2010; Dubow, 2007; Wolters, 2009; Jain, 2011; Tysnes et al., 2010; Asahina et al., 2013; Kaufmann and Goldstein, 2013). Some studies indicated that constipation occurs in PD patients by an average of 10 years or longer before the onset of motor symptoms (Abbott et al., 2001; Kaufmann et al., 2004).

The importance of this problem led to design appropriate studies aimed at assessing non-motor symptoms. Accordingly, specific questionnaires, scores and evaluating scales, such as the NMSQuest (Chaudhuri et al., 2005, 2006, 2010) and the SCOPA-AUT (Verbaan et al., 2007) have been also developed to better define such a neurovegetative involvement. In the recent PRIAMO study, 12 different non-motor symptom domains (gastrointestinal, pain, urinary, cardiovascular, sleep, fatigue, apathy, attention/memory, skin, respiratory, psychiatric, and miscellaneous other symptoms) have been evaluated in a large cohort of PD patients using a semi-structured interview (Antonini et al., 2012). This study showed that the progression of dysautonomic alterations is variable and domain specific, and that it often follows a different pattern compared to motor impairment.

An attempt was also made to predict the severity staging of PD patients using scores of non-motor symptoms. The results of two severity indexes, the Hoehn & Yahr staging for motor aspects and the clinical impression of severity index for PD staging, were classified by means of a machine learning approach. The classification, coupled with a feature subset selection task using an advanced evolutionary algorithm, showed that the appearance of hallucinations, fainting, and inability to control body sphincters or belief in unlikely facts suggests major neurological degeneration (Armañanzas et al., 2013).

Non-motor symptoms have been recently evaluated with the ParkWest Questionnaire also in patients with untreated early PD. The study confirmed the onset of dysautonomic alterations, especially at the gastrointestinal level. Constipation is one of the early symptoms, whereas orthostatic hypotension occurs at a lesser extent (Müller et al., 2011). The "Parkinson's associated risk syndrome" study is aiming to screen a large cohort of subjects to identify those at highest risk for developing PD to be addressed to clinical trials of PD prevention or delay of disease onset, and long-term disability (Akhtar and Stern, 2012).

Among autonomic changes, digestive dysfunctions are a well-recognized feature in PD patients and the entire digestive tract may be affected, with hypersalivation, oropharyngeal and oesophageal dysphagia, gastroparesis, small intestine and colonic dysmotility with constipation, and anorectal dysfunction (Edwards et al., 1991; Byrne et al., 1994; Pfeiffer, 2003; Natale et al., 2008; Lebouvier et al., 2009; Jost, 2010).

Post-mortem and bioptic studies showed the presence of specific alterations in the gut of PD patients. The finding of α-synuclein-positive bodies in myenteric and submucosal plexuses (Ohama and Ikuta, 1976; Kupsky et al, 1987; Wakabayashi et al., 1988; Wakabayashi and Takahashi, 1997), and the loss of tyrosine hydroxylase immunostaining due to a selective dopaminergic neuronal death (Edwards et al., 1992; Singaram et al., 1995; Lebouvier et al., 2008) indicate that PD may begin as a gastrointestinal neurodegenerative disorder which may develop similarly to what described in the central nervous system.

The clinical manifestations in MSA patients may include various non-motor symptoms that arise at disease onset. These symptoms include urinary disorders (frequency, incontinence), erectile dysfunction in men, orthostatic hypotension, sleep disruption, stridor, gastrointestinal alterations (oropharyngeal dysphagia, altered gastric myoelectric activity, severe constipation, fecal incontinence), hypohidrosis, partial ptosis with miosis (Suzuki et al., 2005; Mathias, 2006; Colosimo, 2011; Asahina et al., 2013). A semiquantitative autonomic questionnaire showed that cardiovascular, sudomotor, and pupil alterations were not significantly different between the parkinsonian and cerebellar varieties of MSA, whereas vasomotor, secretomotor, and gastrointestinal changes were more frequent in the parkinsonian variant (Schmidt et al., 2008). A recent prospective multicentre study by the European MSA Study Group confirmed that autonomic failure is a characteristic and common feature in MSA and showed that baseline parkinsonism and neurogenic bladder disturbance are linked to poor survival (Wenning et al., 2013).

The accurate evaluation of the autonomic failure in a prospective study allowed distinguishing PD from MSA, confirming the notion that the primary lesion in PD is both ganglionic and postganglionic, while in MSA it is only preganglionic (Benarroch et al., 2006; Lipp et al., 2009).

Phosphorylated α-synuclein has been shown in the spinal cord, parasympathetic ganglia, vagus nerve, lower oesophagus, and submandibular gland in patients with Lewy body disease (Beach et al., 2010). Furthermore, the presence of Lewy bodies has been identified in the ENS of oesophagus and colon, as well as in the dorsal nucleus of vagus in PD patients (Qualman et al., 1984; Kupsky et al., 1987; Singaram et al., 1995; Wakabayashi, et al., 1999; Beach et al., 2009). An axonal synucleinopathic degeneration (Lewy neurites) was reported in the vagus nerve of a patient with ILBD (Wolters, 2009). As far as the cardiocirculatory system is concerned, PD patients exhibit a "triple whammy": cardiac and extra-cardiac noradrenergic denervation and attenuated baroreflex-cardiovagal function (Jain and Goldstein, 2012). These abnormalities are independent of striatal dopamine depletion and occur very early, preceding the beginning of the motor signs typical of PD (Goldstein et al., 2009; Jain and Goldstein, 2012). Because of the peculiar cardiovascular dysfunction occurring in PD patients, including a severe loss of the noradrenergic innervation, an impairment of the central autonomic control, and drug-induced morpho-functional alterations, Fornai et al. (2007) suggested the term "Parkinsonian Heart".

In PD patients the ^{123}I-*meta*-iodo-benzyl-guanidine (^{123}I-MIBG) scintigraphy, used as an index of sympathetic denervation, showed a reduced accumulation of the tracer in the heart (Takatsu et al., 2000). Since the decreased uptake of ^{123}I-MIBG is detectable before the onset of other autonomic disturbances and is usually not observed in other NDs with parkinsonism, such as MSA, progressive supranuclear palsy or corticobasal degeneration, it is potentially useful in both early and differential diagnosis of PD (Yoshita, 1998; Amino et al., 2008).

Positron emission tomography (PET) after systemic administration of 6-[^{18}F] fluoro-dopamine was also used to visualize cardiac sympathetic innervation, confirming a sympathetic denervation in subjects before the clinical onset of PD (Goldstein et al., 2009; Ziemssen and Reichmann, 2010). Finally, the absence of cardiac spillover of noradrenaline in patients with pure autonomic failure suggested the loss of noradrenergic terminals (Meredith et al., 1991). Investigations carried out on the epicardium from patients with PD and ILBD showed a reduced immunopositivity for thyrosine hydroxylase and the

presence of α-synuclein and Lewy bodies in neurites (Iwanaga et al., 1999; Fujishiro et al., 2008).

In an attempt to predict the onset of motor PD, a clinical study evaluated the role of routine electrocardiograms to monitor the reduced heart rate variability in patients affected by idiopathic rapid eye movement sleep behavior disorder, a population known to be at risk for developing a Lewy body disease, suggesting that impaired heart rate variability may be an early sign of PD (Valappil et al., 2010).

It must be taken into account that only in part cardiovascular changes are directly attributable to PD. As a matter of fact, the dopaminergic replacement therapy also influences autonomic function, as shown by Bouhaddi et al. (2004) who found an impaired cardiac control at mild stages of the disease in newly diagnosed patients who had never undergone treatment, but several cardiovascular changes that were clearly linked to L-dopa therapy.

Orthostatic hypotension associated with supine hypertension, attributable to impaired sympathetic efferent pathways, altered baroreflex mechanisms and dopaminergic therapies, is another important cardiovascular symptom in PD patients. Patients with MSA have intact innervation and can be distinct from PD patients with orthostatic hypotension (Jain and Goldstein, 2012). The *Movement Disorders Society* commissioned a task force to assess existing clinical rating scales addressing symptoms of orthostatic hypotension in PD (Pavy-Le Traon et al., 2011), while Jain and Goldstein (2012) proposed a specific algorithm to distinguish the neurogenic orthostatic hypotension with or without peripheral noradrenergic denervation.

Proposed mechanisms accounting for the orthostatic hypotension in PD include central lesions of the upper brainstem that affect postural control of the blood pressure through baroreflex failure, and cardiac sympathetic denervation, whereas in MSA only central lesions have been implicated. Accordingly, in PD Lewy bodies and neuronal loss were found in both central and peripheral regulatory structures (hypothalamus, parabrachial nucleus, intermediate reticular zone of the medulla, *locus coeruleus* (LC), raphe, parasympathetic Edinger-Westphal and dorsal vagal motor nuclei, intermediolateral cell column, paravertebral and prevertebral autonomic ganglia), whereas in MSA oligodendroglial and neuronal cytoplasmic inclusions were found in brain

regions involved in motor and supraspinal auto-nomic control (Iodice et al., 2011).

Since cytosolic catecholamines are toxic, the decreased vesicular recycling which is observed in Lewy body diseases may be part of a pathogenetic pathway in the catecholaminergic denervation that characterizes these NDs (Jain and Goldstein, 2012). Furthermore, in PD catecholaminergic nuclei are regularly affected. In particular, the noradrenergic LC is damaged, leading to a marked loss of noradrenaline in a variety of brain areas. Moreover, the impairment of the noradrenergic system precedes the dopaminergic damage in the natural time-course of the disease, as demonstrated by biochemical (Tong et al., 2006) and imaging (Sasaki et al., 2006) studies in humans. Genitourinary dysfunctions also impact the quality of life in PD patients, as demonstrated by dedicated questionnaires (Araki and Kuno, 2000; Lemack et al., 2000; Sakakibara et al., 2001). Urinary symptoms mainly consist of urgency, frequency, nocturia and urge incontinence. Nocturia is the most common complaint. Detrusor overactivity was frequently reported in PD patients, whereas detrusor areflexia is an uncommon sign attributed to the use of anticholinergic therapy in PD (Blackett et al., 2009; Sakakibara et al., 2010, 2011; Yeo et al., 2012).

Dopaminergic neurons play an important role in the control of normal micturition. They originate from the ventral tegmental area and project to the pontine micturition centre. The effect on micturition of extrapyramidal structures, such as the globus pallidus, the red nucleus and the substantia nigra, is considered to be inhibitory in nature via D1 dopaminergic receptors. Then, lesions of the basal ganglia may impair detrusor control. In particular, the dopaminergic degenerative loss occurring in PD would result in detrusor overactivity (Yeo et al., 2012).

A decrease in libido and sexual intercourse was found in both sexes, whereas a decrease in orgasm, erection and ejaculation was found in men. Pelvic organ dysfunction appears more severe in the parkinsonian variety of MSA compared to PD. Indeed, Onuf's nucleus is affected in MSA but spared in PD. These anatomical differences would account for the fact that motor PD usually anticipates genitourinary symptoms, whereas in MSA autonomic dysfunctions anticipate motor symptoms (Sakakibara et al., 2001, 2011; Yamamoto et al., 2011; Yeo et al., 2012; Wenning et al., 2013).

The correct recognition of autonomic dysfunctions in PD patients is important in order to design appropriate therapeutic strategies. Only few clinical trials have been performed and the Quality Standards Subcommittee of the American Academy of Neurology addressed this item with a specific report (Zesiewicz et al., 2010). Preliminary reports suggest that fludrocortisone, domperidone, droxidopa or fipamezole may be effective for the treatment of orthostatic hypotension. Botulinum toxin or glycopyrrolate might be used for sialorrhea, whereas macrogol has been proposed to treat constipation. Tropicamide, ipratropium bromide spray, clonidine or radiotherapy are under development for sialorrhea. Sildenafil may be effective for the treatment of erectile dysfunction. Botulinum toxin or behavioral therapy have been suggested for urinary incontinence, and lubiprostone and probiotics for constipation (Seppi et al., 2011; Perez-Lloret et al., 2013).

ALS is traditionally considered a progressive neurodegenerative disease that involves motor neurons, but in recent years a large body of evidence led to the concept of a multisystem degenerative disease. Although infrequent in the early stage, several dysautonomic manifestations also occur in ALS patients, similarly to what happens in PD. Non-motor symptoms and signs concern cardiovascular (blood pressure alterations, tachycardia), digestive (sialorrhoea, dysphagia, delayed gastric emptying, constipation), and urinary (incontinence, neurogenic bladder) systems. Furthermore, reduced lacrimation and altered sudomotor reflex were also reported (Toepfer et al., 1999; Baltadzhieva et al., 2005; Shindo et al., 2011; Yamada et al., 2013).

An enhanced sympathetic and a reduced parasympathetic activity may account for the cardiovascular impairment in ALS. As in PD patients, the MIBG scintigraphy showed a postganglionic sympathetic cardiac denervation in ALS patients (Baltadzhieva et al., 2005).

Experimental models

Since NDs do not naturally occur in animal species, experimental models need to be artificially developed (Levine et al., 2004; Stefanova et al., 2005; Rockenstein et al., 2007; Fernagut and Tison, 2012). Several experimental animal models of NDs

have been designed, but only a few of them have been adopted to study autonomic disorders (Awad, 2011). Nevertheless, it is important to note that although experimental models can recapitulate various aspects of the disease, none of them is completely able to reproduce all the clinico-pathological alterations that occur in each specific ND.

Parkinson's disease

The neurochemical and pathological hallmarks of PD are the dopamine loss in the nigro-striatal system and the presence of Lewy bodies. Since PD is a combination of genetic and environmental factors, many animal models include exposure to toxic agents or genetic manipulation to mimic the symptoms and neuropathology of the disease. While several motor tests have been routinely performed in PD mouse models, including locomotor activity, rotarod, fore-paw stride length, grid test, and pole test, little attention has been paid to investigate non-motor symptoms (Taylor et al., 2010).

Gastrointestinal tract

It is well-known that the enteric nervous system plays an important role in the control of both muscular and secretory gastrointestinal functions (Schemann, 2005; Furness, 2012; Sasselli et al., 2012; Goldstein et al., 2013).

One of the most adopted animal model of parkinsonism is represented by the administration of the neurotoxin 1-methyl, 4-phenyl, 1,2,3,6,-tetrahydro-pyridine (MPTP). The effects of MPTP administration on the gastrointestinal tract of rodents have been described in several studies. Induction of duodenal ulcers, inhibition of duodenal spike activity, decrease in gastric acid and pancreatic secretion (Szabo et al., 1985; Ozdemir et al., 2007), and jejunal disruption of the migrating myoelectric complex (Eaker et al., 1987) were described in rats after a moderate dose of 30 mg/kg MPTP which can elicit peripheral but not central damage. For this reason, the administration of MPTP in rats is not an ideal model. Indeed, MPTP-induced nigro-striatal damage occurs at higher and lethal doses and only the concomitant administration of a peripheral ganglionic blocker would allow the neurotoxin to cause central dopamine decrease in the absence of lethality, but this would conversely limit the ability to act peripherally (Giovanni et al., 1994a,b).

Conversely, the mouse model is more appropriate to reproduce parkinsonism after MPTP administration. An acute inhibition of gastrointestinal motility, measured by the transit of charcoal, was reported in mice, this effect being attributed to both adrenaline and dopamine changes (Haskel and Hanani, 1994). A muscle relaxation in the mouse colon was also observed in an *in vitro* study (Hanani, 1990). However, these effects were measured immediately after MPTP exposure, when the cell degeneration is not yet produced. Therefore, the effects very likely depend on acute noradrenaline release and depletion from peripheral nerve endings. In fact, the neurotoxin produces acutely a non-specific peripheral and central catecholamine release which is followed by stable damage only after a few days (Pileblad and Carlsson, 1988). The MPTP-induced neurotransmitter release, which occurs peripherally immediately after injection, is not related to the subsequent damage (Giovanni et al., 1994a,b; Fornai et al., 1997). This point is important, since it is likely that even the mere damage to the nigro-striatal pathway, in the absence of peripheral toxicity, may contribute to induce parkinsonian constipation (Blandini et al., 2009).

In the study by Anderson et al. (2007) a 40% reduction of thyrosine hydroxylase positive neurons within intestinal plexuses was found at ten days after the administration of a total dose of 60 mg/kg of MPTP in the mouse. However, despite the use of the neurotoxin in the appropriate animal species and the right time window used to analyze the neuropathological effects induced by MPTP, the specific catecholamine affected in the loss of thyrosine hydroxylase-positive neurons was not determined. Paradoxically, MPTP administration was associated with increased enteric motility (Anderson et al., 2007). The propulsive colonic motility must be attributed to the acute administration of the neurotoxin and to the novelty stress of the experiment with the release of stress hormones that are able to stimulate peristaltic enteric reflex in rodents (Wang et al., 2008). As a matter of facts, in another study (Natale et al., 2010) the chronic administration of MPTP in mice induced a persistently delayed colonic motility with constipation, as expected. Furthermore, a reduced thyrosine hydroxylase immunopositivity was shown not only in substantia nigra and striatum, confirming the central dopaminergic neurotoxicity of MPTP, but also in intestinal neurons. The dopaminergic cell loss

was associated with increased α-synuclein levels and reduced DA levels, suggesting that MPTP is a useful model to mimic gastrointestinal damage in PD (Natale et al., 2010).

When chronically administered in monkeys, MPTP was able to increase nitrergic neurons and to reduce tyrosine hydroxylase-positive neurons mainly in the myenteric plexus, whereas cholinergic and vasointestinal peptide-positive neurons and glial cells were unaffected (Chaumette et al., 2009).

Unlike MPTP, the administration of 6-hydroxydopamine (6-OHDA) is more appropriate to induce parkinsonism in rats. When unilaterally injected with 6-OHDA, rats developed a lesion of nigrostriatal dopaminergic neurons associated with a marked reduction of intestinal propulsive activity, this effect being attributed to an impairment of a nitric oxide-mediated descending pathway that controls peristalsis (Blandini et al., 2009). Besides the reduced expression of nitric oxide synthase in the myenteric plexus of distal ileum and proximal colon, in the same animal model an increased presence of vasoactive intestinal polypeptide was found in a subpopulation of nitrergic neurons. Interestingly, a reduced expression of dopamine type 2 receptors in proximal and distal colon was also reported (Colucci et al., 2012). In rats administered 6-OHDA, a delayed gastric emptying and colonic transit was attributed to a significant reduction of nitrergic rather than dopaminergic neurons in the gastrointestinal tract (Zhu et al., 2012).

Gastrointestinal dysfunctions, together with the appearance of inclusions resembling classic Lewy bodies, have been observed in rats following systemic administration of rotenone, another parkinsonism-inducing neurotoxin which inhibits mitochondrial complex I (Drolet et al., 2009). Chronic rotenone treatment in rats also induced an impairment of gastric emptying and longitudinal muscle contraction in response to electrical stimulation, and transiently decreased stool frequency (Greene et al., 2009).

When experimental PD was obtained in rats administered salsolinol (1-methyl-4-phenyl-1,2,3,4-tetra-hydro-isoquinoline), direct effects on interstitial cells of Cajal and changes of the duodenal myoelectrical activity and vagal afferent activity response to gastric distension were reported, suggesting an impairment of the gastrointestinal reflexes encoded within the ENS that resembled delayed gastric emptying, intestinal dysmotility and slow intestinal transit observed in PD patients (Banach et al., 2006). Recent transgenic technologies have contributed to the development of several animal models, many of which recapitulate some features of PD. However, no model has been shown to fully reproduce the symptoms observed in PD patients.

Since transgenic mice over-expressing α-synuclein under Thy1 promoter do not have a reduction of dopaminergic neurons up to 18 months, but were found to exhibit altered colonic functions with decreased basal faecal output and reduced distal colon transit, this model has been proposed to mimic premotor PD (Wang et al., 2008).

In mice with a 95% genetic reduction of vesicular monoamine transporter (VMAT2) expression, apart from a progressive loss of striatal dopamine, L-DOPA-responsive motor deficits, α-synuclein accumulation, and nigral dopaminergic cell loss, several non-motor symptoms have been also observed, including progressive deficits in olfactory discrimination, delayed gastric emptying, altered sleep latency, anxiety-like behavior, and age-dependent depressive behavior. These results suggested that all monoamine transmitter systems (noradrenergic and serotoninergic), not just dopamine, play a role in the clinical manifestations of PD and that this approach appears more reliable to study non-motor symptoms (Taylor et al., 2009).

Considering the interesting hypothesis that environmental agents or pathogens might invade the digestive tract and retrogradely affect the vagus nerve to reach brain nuclei (Braak et al., 2003), multiple injections of a selective proteasome inhibitor (PSI) into the rat gastric wall were able to induce the appearance of α-synuclein-immunopositive intracytoplasmatic inclusions, but not neuronal death, in the dorsal motor nucleus of the vagus nerve. These data indicate that the mere dopaminergic degeneration induced by neurotoxins, such as MPTP, may be insufficient to reproduce such an alteration in PD, and that genetic defects responsible for dysfunctioning of the UPS can play an important role in the onset of early PD (Miwa et al., 2006).

Different animal species, different toxic agents, and different doses and experimental protocols might explain the conflicting data coming from animal models of parkinsonism. Furthermore, it must be taken into account that in the enteric nervous system

dopaminergic neurons represent only a small portion of the neuronal population, and that many other enteric non-dopaminergic neurons could also be damaged during the progression of PD. Finally, the occurrence of neuroplasticity processes can not be excluded in the gut after a specific neuronal damage (Goetze and Woitalla, 2008; Chaumette et al., 2009).

Cardiovascular system

Some studies focused on setting up experimental models addressed to investigate peripheral alterations of the cardiovascular system in PD. In MPTP-treated mice, the cardiac noradrenergic denervation due to the loss of noradrenergic axons directed to the heart that occurs in PD patients, was reproduced. Accordingly, in this animal model the cardiac accumulation of ^{123}I-MIBG was significantly reduced, similarly to what observed in PD patients (Takatsu et al., 2000, 2002; Fukumitsu et al., 2006, 2009). In an *in vitro* study, a marked blockade of ^{123}I-MIBG uptake was found in a cultured pheochromocytoma cell line (PC-12) pretreated with MPTP, suggesting that cardiac sympathetic nerves are primarily impaired in PD, despite the presence or absence of systemic autonomic failure (Takatsu et al., 2002).

Furthermore, cardiac noradrenaline (Fuller et al., 1984; Fukumitsu et al., 2006, 2009; Amino et al., 2008; Ruffoli et al., 2008a) and dopamine (Amino et al., 2008) concentrations and norepinephrine transporter (NET) density in postganglionic cardiac sympathetic nerve terminals (Fukumitsu et al., 2006, 2009) were also found decreased in MPTP-treated mice. In particular, a decrease in cardiac noradrenaline concentrations, associated with reduced mean arterial blood pressure, was also reported in rats treated with MPTP (Fuller et al., 1984). However, despite a cardiac noradrenaline reduction, in MPTP-treated mice Amino et al. (2008) did not find a significant decrease in NET, synaptophysin and tyrosine hydroxylase of noradrenergic nerve terminals when examined by immunohistochemistry or western blot analysis. It was concluded that preservation of cardiac nerve terminals and their functional loss suggest that the mechanism of toxicity induced by MPTP to noradrenergic neurons in the cardiac sympathetic nervous system may be somewhat different from that to dopaminergic neurons in substantia nigra (Amino et al., 2008).

Electrophysiological studies carried out on cardiac ventricular myocytes isolated from mice treated with MPTP showed that the contractile properties were significantly altered, with decreased cell shortening, prolonged duration of relaxation, and reduced velocities of both shortening and relengthening, suggesting a reduced adrenergic responsiveness. Indeed, other abnormalities were observed, including a 67% reduction of beta-adrenergic receptor expression in myocardial membranes, a reduced peak of intracellular calcium ion sequestration and sarcoplasmic reticulum calcium ion load, and a reduced response to noradrenaline and isoproterenol (Ren et al., 2004).

Apart from cardiac sympathetic denervation, baroreflex failure with consequent orthostatic hypotension is another important early complaint in PD patients. In a recent study, the baroreceptor reflex control of heart rate was assessed in mice overexpressing human wild-type alpha-synuclein, a genetic mouse model of synucleinopathy, where tachycardia following nitroprusside-induced hypotension or muscarinic blockade with atropine was significantly reduced, showing that this autonomic dysfunction of PD occurs prior to dopaminergic damage in striatum (Fleming et al., 2013).

Taken together, these findings suggested that the postganglionic sympathetic nerves may be damaged by MPTP in experimental models of PD during the early stage, because dopaminergic neurons and sympathetic nerves are substantially similar in their plasma membrane transporter (Takatsu et al., 2000). It would be not surprising to find a severe damage in the cardiovascular system, considering that MPP+, the neurotoxic metabolite of MPTP, reaches its highest concentrations in the mouse heart (Fuller et al., 1988). The noradrenergic impairment occurring in PD is also confirmed by the histopathological observation that LC is damaged, as demonstrated by preclinical studies in various animal species (Mavridis et al., 1991; Gesi et al., 2000). Furthermore, Fornai et al. (2007) showed the impact of noradrenaline deficit on the survival of nigral dopaminergic neurons.

Male reproductive system

In mice treated with MPTP for 7 days, apart from a significant nigrostriatal dopamine depletion, a marked decrease in testosterone plasma levels and Leydig cell loss were observed. In particular, ultrastructural investigations showed mitochondrial

degeneration in spared Leydig cells. Furthermore, the morphology of the interstitium appeared altered, with a marked decrease in tyrosine hydroxylase positivity in the interstitium. These changes were also accompanied by a significant decrease in noradrenaline testicular levels (Ruffoli et al., 2008b). Considering some common features between PD and ALS, it is not surprising that in an ALS transgenic mouse model carrying the G93A mutant form of the human SOD1 transgene, some autonomic alterations were found, including higher heart rate at rest, decreased mydriasis following administration of morphine and lower rectal temperature during cold stress and following morphine administration (Kandinov et al., 2011).

Summary

Neurodegenerative diseases (NDs) include a large variety of disorders that affects specific areas of the central nervous system, leading to psychiatric and movement pathologies. Non-motor alterations also occur in several viscera, in particular the gastrointestinal tract and they often precede the onset of motor symptoms by several years. For this reason, dysautonomic changes can be predictive of NDs and their correct recognition is being assuming a remarkable importance. This peculiar feature led to the concept that neurodegeneration may initiate in the periphery and propagate retrogradely towards the central nervous system in a prion-like manner. In recent years, a particular attention was dedicated to the clinical assessment of autonomic disorders in patients affected by NDs. In this respect, experimental animal models developed to understand the neurobiology underlying these effects are summarized in the review.

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