

Review

Cardiovascular Autonomic Dysfunction in Animal Models of Parkinson's Disease

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Abstract. Cardiovascular autonomic dysfunction is a common non-motor symptom associated with synucleinopathies such as Parkinson's disease (PD). Several recent clinical studies indicate that cardiovascular autonomic impairments including orthostatic hypotension and sympathetic denervation may precede the development of the cardinal motor symptoms in PD, making cardiovascular dysfunction an attractive target for the development of biomarkers for early detection and potential neuroprotective strategies for PD. However, the pathologic mechanisms underlying cardiovascular dysfunction as well as many of the non-motor symptoms in PD remain unknown. This is likely due, in part, to an initial under-appreciation of PD as a systemic disorder as well as limited research in cardiovascular dysfunction in animal models of PD. Here, we highlight studies that have investigated cardiovascular dysfunction in rodent models of PD and the potential usefulness of genetic mouse models of PD for this endeavor.

Keywords: Alpha-synuclein, orthostatic hypotension, sympathetic denervation, baroreflex, heart rate variability, 6-hydroxydopamine, MPTP

SYMPATHETIC AND PARASYMPATHETIC NERVOUS SYSTEM DYSFUNCTION IN PARKINSON'S DISEASE

The interaction between the sympathetic and parasympathetic divisions of the autonomic system plays a critical role in control of heart rate and blood pressure with alterations in either system leading to impaired cardiovascular function. The sympathetic division is important for mobilizing the body for selective energy expenditure and is associated with the “fight or flight” response. In the brainstem the rostral ventrolateral medulla regulates the sympathetic nervous system. Sympathetic activation results in increased heart rate and blood pressure, pupil dilation, and sweating. Norepinephrine is the main neurotransmitter related with increased heart rate

and blood pressure (acetylcholine is associated with increased sweating). In contrast, the parasympathetic division is related with reduced energy expenditure and increased energy conservation, “rest and digest”. The vagus nerve drives parasympathetic activation and promotes gastrointestinal processes, fat storage, and pupil constriction. Acetylcholine is the main neurotransmitter of the parasympathetic division. Both systems interact with the baroreceptor reflex to tightly regulate blood pressure.

Impaired autonomic control of cardiovascular function is associated with neurodegenerative disorders such as the synucleinopathies. Synucleinopathies are diseases characterized by the development of alpha-synuclein containing Lewy bodies and neurodegeneration and include Parkinson's disease (PD), multiple system atrophy, and dementia with Lewy bodies [1]. In PD, the most common synucleinopathy, impairments in cardiovascular autonomic function are frequently observed and manifest in a range of cardiovascular alterations in patients (Table 1). The noradrenergic aspect of the sympathetic nervous

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Table 1
Cardiovascular autonomic dysfunction in PD

Synucleinopathy	Cardiovascular autonomic dysfunction	Associated pathology
Parkinson's disease	Orthostatic hypotension, syncope, impaired baroreflex, reduced heart rate variability, post-prandial hypotension, hypertension while supine	Post-ganglionic lesions, Cardiac sympathetic denervation, abnormal alpha-synuclein accumulation in sympathetic ganglia, Lewy bodies in periphery (stellate ganglia and cardiac plexus) and centrally (locus coeruleus, medulla, hypothalamus)
Multiple System Atrophy	Orthostatic hypotension, post-prandial hypotension, supine hypertension	Pre-ganglionic sympathetic lesions
Dementia with Lewy Bodies	Syncope, Orthostatic hypotension	Sympathetic denervation, Cardiovagal denervation

system is particularly affected in PD. For example, profound sympathetic noradrenergic denervation has been shown functionally by measuring myocardial ^{123}I -metaiodobenzylguanidine (MIBG, an analogue of norepinephrine) and 6- ^{18}F fluorodopamine uptake in peripheral adrenergic nerves in PD patients [2, 3]. This has been verified anatomically through tyrosine hydroxylase immunohistochemistry of postganglionic cardiac sympathetic nerves of PD patients [4]. Interestingly, this decrease in tyrosine immunoreactivity in postganglionic cardiac sympathetic nerves is not seen in multiple system atrophy, another synucleinopathy with symptoms of autonomic dysfunction, and provides a method to differentiate the two diseases, which can be difficult to do based on symptoms, especially in the early stages [4]. Autonomic tests such as the Valsalva maneuver and head up tilt can detect baroreflex failure and orthostatic hypotension in PD patients [5–7]. Orthostatic hypotension, an uncompensated fall in systolic blood pressure after assuming an upright position, is reported to occur in ~10–40% of PD patients and is associated with progressive cardiac sympathetic denervation [3, 8]. Orthostatic hypotension is a particularly dangerous condition in PD because it can contribute to falls which patients are already susceptible to. Furthermore, orthostasis is notoriously under-reported by patients having unexplained falls in PD. Alterations in heart rate variability measures have also been observed in PD [9, 10] and electrocardiographic assessment of heart rate variability has been proposed as a potential screen for those at risk of developing PD [11].

EARLY SYMPTOMATOLOGY

Recent clinical studies suggest that many of the non-motor symptoms associated with PD may develop early in the disease, prior to the onset of the cardinal motor symptoms. Indeed, neurocardiological alterations have been reported to occur early in several of the synucleinopathies [12–15]. For example, both

orthostatic hypotension and cardiac sympathetic denervation have been shown to develop before diagnosis of PD in some cases [15, 16]. Both Orimo et al. [17] and Fujishiro et al. [18] found that cardiac sympathetic denervation and alpha-synuclein pathology occur in Incidental Lewy Body disease, which is thought to reflect pre-manifest PD [19]. In addition to their early development, cardiovascular alterations have also been linked with other non-motor symptoms including olfaction, cognition, and sleep dysfunction [20–23]. This suggests cardiovascular autonomic dysfunction could be a useful biomarker for detecting the early stages of the disease as well as a target for potential therapeutics.

PERIPHERAL AND CENTRAL NERVOUS SYSTEM PATHOLOGY

While synucleinopathies are typically characterized by neuronal degeneration of specific cell types such as nigrostriatal dopamine neuron loss in PD, there is considerable widespread central and peripheral nervous system pathology that likely contributes to the non-motor symptoms such as cardiovascular autonomic dysfunction [19, 24–26]. In addition to nigrostriatal neuronal death in PD, there is also cell loss in the catecholaminergic C1 region in the ventrolateral medulla and in noradrenergic neurons in the locus coeruleus, areas important for blood pressure regulation [27]. Lewy bodies that contain alpha-synuclein are observed in central and peripheral regions associated with cardiovascular autonomic function such as the medulla, locus coeruleus, cardiac plexus, and stellate ganglia [19, 24–27]. Furthermore, patients with a familial form of PD associated with duplication or triplication of the alpha-synuclein locus show impaired parasympathetic function in the Valsalva maneuver and reduced cardiac sympathetic innervation suggesting an important role for alpha-synuclein in the pathogenesis of sympathetic and parasympathetic dysfunction in PD [28, 29]. Mechanistically, Orimo et al. [30] showed in

Table 2
Cardiovascular autonomic dysfunction in rodent models of PD

Model	Dysfunction	References
6-OHDA	↓ nocturnal heart rate, attenuated baroreflex response	[37, 44]
MPTP	Reduced ¹²⁵ I-MIBG uptake, NE turnover, NE transporter density, preserved myocardial nerve fibers, depressed contractility	[46, 47, 49, 50]
Overexpression of Alpha-Synuclein in Mouse	↓ NE-induced contraction (aortic ring preparation), Impaired baroreceptor control of heart rate	[62, 75, 76]

sympathetic ganglia from PD patients that degeneration of the cardiac sympathetic nerves precedes neuron loss in parvertebral sympathetic ganglia and that pathological alpha-synuclein is found in the distal axons in the earlier stages of the disease and as the disease progresses pathological alpha-synuclein can be found more in the soma [30]. Most recently the same group showed that unmyelinated axons of cardiac nerves are more susceptible to degeneration than myelinated axons and this is also associated with more accumulation of alpha-synuclein in unmyelinated axons [31].

CARDIOVASCULAR AUTONOMIC DYSFUNCTION AND ANIMAL MODELS OF PD 6-HYDROXYDOPAMINE (6-OHDA)

The toxicity of 6-OHDA is specific to catecholaminergic cells and is widely used to create animal models of Parkinson's disease [32–35]. 6-OHDA injected into the striatum, substantia nigra pars compacta, or medial forebrain bundle results in the destruction of dopaminergic neurons [32, 36]. The norepinephrine uptake blocker desipramine is often administered prior to nigra and medial forebrain bundle injections to create a selective dopaminergic lesion. These selective lesions have been used to study whether nigrostriatal dopamine cell loss can disrupt cardiovascular autonomic function. Indeed, intracerebral (medial forebrain bundle) administration of 6-OHDA in the rat does attenuate striatal dopamine release following acute increased blood pressure (phenylephrine administration) or decreased blood pressure (carotid occlusion) and reduces baroreflex sensitivity [37]. Sinoaortic denervation in the rat, a technique that has been used to study the role of the baroreflex in cardiovascular regulation [38], has also been used to study whether disruption of the baroreflex can enhance toxicity of specific PD-related neurotoxins such as 6-OHDA and rotenone. While sinoaortic denervation does not potentiate the effects of intracerebral 6-OHDA or systemic rotenone in the rat, sinoaortic denervation does lead to

decreased tyrosine hydroxylase activity in the striatum and substantia nigra [39–41]. Interestingly, injections of 6-OHDA to the neighboring ventral tegmental area alters circadian blood pressure regulation [42, 43]. Similarly, decreased nocturnal heart rate was observed in rats with unilateral 6-hydroxydopamine medial forebrain bundle lesions [44]. The 6-hydroxydopamine studies suggest a reciprocal relationship between mid-brain dopamine pathways and cardiovascular parameters including blood pressure and heart rate (Table 2).

1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP)

Similar to 6-OHDA, MPTP is a neurotoxin commonly used to model aspects of PD in animals. Systemic administration of MPTP results in reduced cardiac norepinephrine in rats and mice [45]. More recently Fukumitsu et al. showed reduced ¹²⁵I-meta-iodobenzylguanidine uptake, norepinephrine turnover, and norepinephrine transporter density in mice treated with MPTP [46–48]. Studies in MPTP-treated mice also show preserved myocardial nerve fibers and altered cardiac contractile function [49, 50]. These studies highlight that MPTP in the mouse not only alters central catecholamine systems it also has a profound effect on peripheral cardiac monoamine systems as well (Table 2). In contrast, in non-human primates, MPTP induces phasic peripheral catecholaminergic dysfunction followed by full recovery [51].

GENETIC MOUSE MODELS

With the discovery over the past 14 years of novel genes associated with familial forms of PD has come the development of novel genetic mouse models of Parkinsonism. These models may be particularly useful for studying the non-motor symptoms of PD and potential early markers of the disease. Cardiovascular autonomic dysfunction has been identified in PD patients with mutations or multiplication of the alpha-

Table 3
Heart rate responses (bpm) in awake wildtype and Thy1-aSyn mice

	PE		SNP		AT	
	Base	Drug	Base	Drug	Base	Drug
Wildtype	456 ± 15	340 ± 29	486 ± 48	702 ± 9 ^Δ	514 ± 24	658 ± 7 ^Δ
Thy1-aSyn	544 ± 65	441 ± 31	552 ± 31	552 ± 47*	591 ± 25	605 ± 26

Wildtype and Thy1-aSyn mice were equipped with arterial telemetry at 3–5 months of age ($n = 3–5$ per genotype). bpm = beats per minute, PE = phenylephrine (3 mg/kg, ip), SNP = sodium nitroprusside (1 mg/kg, ip), AT = atropine (1 mg/kg, ip). ^Δrepresents $p < 0.05$ compared to wildtype baseline, *represents $p < 0.05$ compared to wildtype drug, 2×2 mixed design ANOVA, Fisher's LSD post hoc.

synuclein gene and in patients with mutations in the leucine-rich repeat kinase 2 gene [52–54].

While the presynaptic protein alpha-synuclein is associated with both sporadic and familial forms of the PD the function of alpha-synuclein remains unclear [55–59]. In the brain it is involved in synaptic function, vesicular handling, and synaptic plasticity [60, 61]. Work by Braak and colleagues suggests alpha-synuclein pathology in brain regions outside of the substantia nigra, such as the medulla, locus coeruleus, and olfactory regions, may precede the development of alpha-synuclein pathology in the substantia nigra and provides a potential link between non-motor impairments and alpha-synuclein pathology in PD [19].

Mice overexpressing wildtype or mutated forms of alpha-synuclein may be particularly promising models to study cardiovascular autonomic dysfunction given the recent work by Orimo and colleagues highlighting the development of pathological alpha-synuclein accumulation and cardiac sympathetic denervation [30, 31]. Alpha-synuclein is found in mouse sympathetic nerves innervating the aorta and is associated with contractility suggesting alpha-synuclein mouse models may be useful in elucidating potential underlying mechanisms of cardiovascular dysfunction in PD [62].

There are currently numerous mouse models of alpha-synuclein overexpression and while most do not develop the characteristic loss of nigrostriatal dopamine neurons several do show non-motor impairments such as gastrointestinal dysfunction, cognitive dysfunction, and altered emotional reactivity [63–67]. However, most have not assessed or found cardiovascular autonomic dysfunction in mutant mice. For example, Kuo et al. [67] showed autonomic abnormalities in the enteric nervous system of mice with A53T and A30P mutations in alpha-synuclein but these mice did not develop cardiovascular dysfunction.

Based on the compelling relationship between alpha-synuclein pathology and cardiovascular dysfunction in PD we have begun to characterize mice overexpressing wildtype, human alpha-synuclein under the Thy1 promoter (Thy1-aSyn) [68]. These

mice show increased alpha-synuclein levels throughout the brain and develop proteinase K-resistant alpha-synuclein aggregates in several brain regions, including the substantia nigra, locus coeruleus, and olfactory bulb [68–70]. Thy1-aSyn mice show progressive impairments in sensorimotor function and non-motor impairments reminiscent of those observed in PD such as deficits in gastrointestinal function, olfaction, cognitive, and neuropsychiatric dysfunction [70–73]. By 14 months, these mice show a significant decrease in striatal dopamine and L-Dopa responsive behavioral deficits, indicating they reproduce important characteristic features of PD [74]. This suggests these mice may have functional impairments in cardiovascular autonomic function similar to those observed in PD. We measured baroreceptor control of heart rate in both anesthetized and awake Thy1-aSyn and wildtype mice in response to transiently increased and decreased blood pressure. Mice were administered the alpha 1-adrenergic agonist phenylephrine, the nitric oxide donor sodium nitroprusside, and the muscarinic antagonist atropine and blood pressure and heart rate responses were recorded. In response to the vasodilator sodium nitroprusside, anesthetized and awake wildtype mice displayed the characteristic tachycardic response to the sudden drop in blood pressure, while Thy1-aSyn mice showed no significant change in heart rate during an equivalent transient decrease in systolic blood pressure [75–76]. Similarly in PD patients, a blunted heart rate increase has been reported in response to the feet down tilt maneuver [12, 77]. In addition to nitroprusside, Thy-aSyn mice showed a similar abnormal response to atropine, where heart rate increased in wildtype but not in Thy1-aSyn mice (Table 3). These findings are important because they provide a foundation for future studies aimed at identifying brain structures and pathological mechanisms associated with cardiovascular autonomic dysfunction in PD. In addition, these results underscore the usefulness of alpha-synuclein overexpressing mice in the study of non-motor symptoms associated with PD.

CONCLUSION

Cardiovascular autonomic dysfunction is a common non-motor symptom seen in the synucleinopathies. The majority of studies aimed at characterizing and understanding the pathology associated with cardiovascular dysfunction in these diseases has been performed primarily in patients and surprisingly work in animal models has been sparse. However, given the high prevalence of these symptoms and the potential early development of cardiovascular autonomic dysfunction in synucleinopathies, studies in animal models could be particularly valuable at this point. Combinations of toxin, genetic, and viral animal models would allow mechanistic approaches to understanding the etiology of cardiovascular autonomic dysfunction. In addition, models that display multiple non-motor symptoms would be useful in understanding the relationship between symptoms that are correlated with each other such as olfaction and cardiovascular dysfunction. Furthermore, nonmotor symptoms that are unmet medical needs, such as cardiovascular autonomic dysfunction, are important targets for novel treatments and thus, make these models useful for pre-clinical studies of novel treatments for PD.

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