

REVIEW

Molecular, Neurochemical, and Behavioral Hallmarks of Reserpine as a Model for Parkinson's Disease: New Perspectives to a Long-Standing Model

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Abstract

The administration of reserpine to rodents was one of the first models used to investigate the pathophysiology and screening for potential treatments of Parkinson's disease (PD). The reserpine model was critical to the understanding of the role of monoamine system in the regulation of motor and affective disorders, as well as the efficacy of current PD treatments, such as L-DOPA and dopamine agonists. Nevertheless, with the introduction of toxin-induced and genetic models of PD, reserpine became underused. The main rationale to this drawback was the supposed absence of reserpine construct validity with PD. Here, we highlight classical and recent experimental findings that support the face, pharmacological, and construct validity of reserpine PD model and reason against the current rationale for its underuse. We also aim to shed a new perspective upon the model by discussing the main challenges and potentials for the reserpine model of PD.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. Its onset is rarely before the age of 50 years and a sharp increase of the incidence occurs after the age of 60 years (19). PD affects approximately 1%–2% of the population over the age of 60 (63), with a higher prevalence in men than in women (19, 62). Most importantly, it is a disorder with progressive onset and escalating deterioration of quality of life (28). Therefore, PD is a social and economic burden to countries with increasing life expectancy, and for this reason, the scientific interest in the disorder is continuously emphasized.

PD diagnosis is based on its cardinal motor symptoms, which include bradykinesia, rigidity, resting tremor, and postural instability (108). However, even though PD is essentially a motor disorder, patients present equally incapacitating nonmotor symptoms. Furthermore, those symptoms may appear previously or concomitantly to motor symptoms (126) and include sleep disorders (83, 134, 152), anxiety (154), depression (15, 97), neuropathic pain and nociceptive sensitization (27, 72, 196), impulsivity (160, 203,

204), dementia and executive function impairment (1, 7, 49, 123), olfactory dysfunction (7, 60), and constipation (48, 152).

The motor alterations are a consequence of dopaminergic neuronal loss in the substantia nigra (SN) (92, 108), where the main dopaminergic projection to the motor-regulating nucleus in the basal ganglia originates (52, 120). Nonetheless, loss of dopaminergic neurons in the ventral tegmental area (VTA)—projecting to limbic areas and to prefrontal cortex—is also reported in PD (192, 197). This loss results in emotional and cognitive deficits (154, 165). Furthermore, other neurotransmission disturbances are described, as revealed by histopathological markers in serotonergic (101, 194), noradrenergic (28, 211, 213), and cholinergic (197, 211) neurons.

Studies have also characterized the neurochemical alterations in PD at the cellular and genetic levels. Five to 10% of PD cases are traced to familial heritage and studies have identified some genes that underlie rare familial forms of the disease (206). This approach highlighted genes involved in cellular pathways implicated in synaptic function (*SNCA*: α -synuclein), ubiquitin-proteasome protein degradation (*Parkin* and *UCHL1*), respiratory chain (*PINK1*), protein phosphorylation (*LRRK2*), and oxidative

stress response (*DJ-1*) (59, 163, 202, 206). Hence, impairment of these pathways leads to oxidative stress and defective protein folding, signaling, and degradation (47, 104, 114, 184). Finally, the accumulation of defective protein aggregates—mainly constituted by α -synuclein, parkin, and ubiquitin, known as Lewy's bodies (200)—is followed by cell death. Thus, the pathogenesis of PD primarily relates to the generation of oxidative stress and accumulation of defective proteins.

The genetic alterations are in accordance with epidemiological associations to PD. These associations comprise exposure to environmental toxins that act on the respiratory chain (42, 143, 195)—such as pesticides, heavy metals, and carbon monoxide—and neuroinflammation (88, 200). Both events result in the generation of toxic reactive oxygen (ROS) and reactive nitrogen species, giving rise to cell damage and eventually cell death. In brief, PD harbors the oxidative imbalance as a common molecular pathway to cellular stress and neurodegeneration. Thus, animal models of PD aim to reproduce the aforementioned cellular and molecular damages (44, 61, 129), while clinical and preclinical therapeutic strategies target different candidate steps of these pathways to slow PD progression (34, 91).

ANIMAL MODELS OF PD

Current studies use genetic and neurotoxic approaches to reproduce pathophysiological hallmarks in animal models of PD. In genetic studies, some strategies focus on the overexpression of normal or truncated autosomal dominant genes, such as *SNCA* (23, 105, 137, 205) and *LRKK2* (117, 118), and knockout or knockdown of autosomal recessive genes, as *Parkin*, *PINK1*, or *DJ-1* (106, 107, 157, 191). Nevertheless, none of these strategies recapitulates the key clinical and neuropathological features of PD and they only account for 5%–10% of PD cases (206). As a result, the most frequently used strategy is to induce oxidative imbalance and dopamine (DA) depletion by the administration of toxins or drugs that act upon dopaminergic neurons (37, 44, 61, 71, 129, 136, 167, 177, 210).

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) are the most used toxins in animal models of PD because of their rather selective actions upon dopaminergic neurons (9, 18, 61, 129). Both enter the dopaminergic neuron by the DA transporter (DAT) and inhibit the complex I in the respiratory chain, causing adenosine triphosphate (ATP) reduction, oxidative damage, protein aggregation, cell death, and DA depletion (61, 94, 129, 181). MPTP is a highly lipophilic prototoxin that readily crosses the blood–brain barrier when peripherally administered (161). Once in the brain, MPTP is converted by glial monoamine oxidase (MAO)-B into its intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium, which is rapidly oxidized into 1-methyl-4-phenylpyridinium and then reabsorbed by the dopaminergic neuron through the DAT (45). A disadvantage of this model is that rodents are more resilient to cell damage induced by MPTP compared with primates. This results in the need for higher dosages and increased variability in neurodegeneration within treated animals (43, 61, 170). In addition, there is a high risk of contamination to researchers because of the handling of large doses of MPTP and the respective biological waste (155).

6-OHDA, on the other hand, does not cross the blood–brain barrier and is directly administered into the brain (18, 26, 61, 170).

Contrastingly from MPTP, 6-OHDA enters noradrenergic neurons as well, through the noradrenaline (NA) transporter (NAT) (29). This lack of specificity is usually resolved by the coadministration of inhibitors of NA and serotonin (5-HT) reuptake, such as nortriptyline or desipramine (27, 56, 188). Although safer regarding contamination risk compared to MPTP, bilateral administration of 6-OHDA results in extensive neuronal loss and severe motor impairment followed by death. After administration, animals need tube-feeding because of aphagia and adipsia (55, 198). In order to avoid these issues, most studies perform the unilateral lesion with 6-OHDA and assess motor deficit by inducing unilateral rotating behavior with dopaminergic agonists (171, 188). Although rotational behavior lacks face validity with PD (55), some studies evaluate forelimb akinesia (evaluated by adjusted stepping and limb-use asymmetry tests) after unilateral 6-OHDA administration (145, 169, 183). Nevertheless, even though the forelimb akinesia provides face validity, the unilateral lesion is still a weak approach to mimic PD pathology and symptomatology.

Alternatively, studies have employed environmental toxins such as rotenone, paraquat and maneb to model PD in rodents (9). Of those, rotenone is the most used because of its lipophilic structure, easiness to cross biological membranes, ability to inhibit complex I, and generate ROS (16, 93, 172). However, despite its close relationship to epidemiological risk factors of PD, rotenone's lack of selective action results in systemic and peripheral toxicity (74, 151, 158) and highly variable dopaminergic lesions (22, 43, 172, 212).

Finally, the administration of reserpine—an inhibitor of the vesicular transporter of monoamines in the central nervous system (VMAT2)—was one of the earliest animal models of PD. Reserpine is an alkaloid extracted from *Rauwolfia serpentina* and was first used as a potent antihypertensive drug because of its capacity to deplete cellular monoamine content (76, 125, 150). The clinical use of reserpine led to the observation that patients chronically treated with reserpine developed lethargy, depression, and motor dyskinesia, implicating the monoamine system in the pathophysiology of affective and motor disorders (76, 102). Readily after, reserpine was used in rodents to mimic parkinsonian motor and nonmotor impairments (17, 38, 39, 51, 69, 164, 175). Although considered outdated in comparison with the aforementioned models, the reserpine model mimics key features of PD symptomatology, neurochemistry, and pharmacology. For this reason, the model was useful to elucidate the relevance of dopaminergic neurotransmission to motor control as well as to screen for candidate drugs for treatment of PD. This review will highlight a new perspective upon the model and reason against the current rationale for the undervaluation of the reserpine-induced parkinsonism model.

MOTOR AND NONMOTOR BEHAVIORAL IMPAIRMENT IN THE RESERPINE MODEL

The relationship between reserpine and PD was first reported by Carlsson *et al*, who observed that the akinetic state induced by reserpine in rodents was alleviated by L-DOPA (38, 39). At doses varying from 1 to 10 mg/kg, reserpine induces a wide range of motor impairments that resemble PD, mainly akinesia, hypokinesia, catalepsy, limb rigidity, and oral tremor (17, 51, 164). These motor features are a consequence of the blockage of

VMAT2 (201), leading to total monoamine depletion, including DA, NA, and 5-HT.

Besides the typical motor impairment, reserpine is also able to produce aversive (70, 174) and recognition (167) memory deficits, anxiety-like behavior (25, 112), depressive and anhedonic-like behaviors (10, 11, 175), and nociceptive sensitization (10, 11, 119, 144). Moreover, the memory impairment and the anxiety-like behavior were described in a dose range (0.1–0.5 mg/kg) that did not produce motor impairment (25, 70, 167, 174). This outcome allowed the dissociation of an important confounding factor in behavioral analyses.

More recently, the repeated treatment with low doses of reserpine (0.1 mg/kg) has been suggested as a progressive model of PD (71, 167). Under this treatment regimen, animals progressively developed motor impairment in the open field, catalepsy bar, and oral movement tests after repeated injections of a low dose (0.1 mg/kg) of reserpine. Deficits in these motor tests recapitulate main motor symptoms of PD, such as hypokinesia and bradykinesia, in the open field and catalepsy bar test (ie, slowness and difficulty to initiate movements) and resting tremor in the oral movement test.

In the aforementioned study (167), the motor impairments were preceded by cognitive impairment in the novel object recognition task. This impairment was also accompanied by neuronal alterations compatible with the pathophysiology of PD such as reduction in tyrosine hydroxylase (TH) immunostaining (167) and increased lipid peroxidation in the striatum (71). Furthermore, the object recognition index positively correlated with VTA immunostaining for TH, suggesting neuronal pathways disruption other than the nigrostriatal pathway playing an important role in nonmotor symptoms of PD. In addition, the object recognition deficit occurred after a 1-h interval between training and test sessions (167), but not when the two sessions were 24-h apart (71). In other words, reserpine-treated rats presented short-term, but not for long-term, memory deficit previously to motor deficits. Thus, performance in the task requires recognition and executive functions. These findings are in accordance with early PD symptomatic description, as executive function, attention deficit and episodic and procedural memory impairment have been described (20, 64, 115, 160, 162, 204). Furthermore, acute administration of low dose of reserpine resulted in emotional processing deficits in aversive memory tasks, such as context conditioning (70) and discriminative avoidance (40) task, but not motor impairment. In parallel, immobility in the forced swim test correlated with pain indexes, indicating a comorbid relationship between different reserpine-induced nonmotor symptoms (10). Similarly, PD nonmotor impairments comprise anxiety (154), depression (15, 97), and nociceptive sensitization (30, 72, 196). Thus, nonmotor findings induced by reserpine resemble nonmotor PD symptoms, reinforcing reserpine's face validity as a PD model.

PHARMACOLOGICAL AND PREDICTIVE QUALITY OF THE RESERPINE MODEL

The use of reserpine was critical to the first demonstration of the therapeutic efficacy of L-DOPA (38, 178). This effect was shortly after observed in humans (54) and the reserpine model was established for screening of potential symptomatic treatment efficacy of new drugs for PD. Indeed, besides L-DOPA, the reserpine model

predicted other current symptomatic anti-Parkinson treatments: apomorphine (85), pramipexole (68, 122), ropinirole (77), rotigotine (199), pergolide (51, 98), bromocriptine (98, 99), and cabergoline (133). Likewise, reserpine-induced motor impairment is also reversed by agents that are used in association with L-DOPA, for example: muscarinic antagonists, such as benzotropine and trihexyphenidyl (85); MAO-B or catechol-O-methyltransferase (COMT) inhibitors, such as selegiline (51, 176), rasagiline (73), and tolcapone (121); and amantadine (51, 53, 85, 100, 176). Table 1 summarizes different types of motor impairment induced by reserpine that are reversed by these drugs. In fact, reserpine is still currently used to assess anti-parkinsonian efficacy of novel agents, such as D3 receptor agonists (80), inhibitors of glutamate release (103), group III metabotropic glutamate receptor agonists or positive allosteric modulators (14, 32, 142), group I muscarinic metabotropic receptor antagonists or allosteric modulator (207), and mixed adenosine A2A/A1 antagonists (13, 173).

Reserpine is also employed in the screening for antioxidant and anti-inflammatory treatments to prevent motor impairments such as dyskinesia (5, 10, 24, 66, 139, 147, 148). Current literature on oral dyskinesia implicates oxidative stress on the pathophysiology of the disorder (3, 4, 136, 186, 187). Accordingly, monoamine depletion in reserpine-treated rats is followed by increase of reactive oxygen and nitrogen species and cell damage (179). The metabolism of catecholamine (CA) intrinsically results in ROS formation, which is increased as a consequence of free CA in the cytoplasm of reserpine-treated rats (127, 156). Thus, oxidative stress and cell damage sums up to the monoamine depletion to impair motor performance. For this reason, treatment with antioxidants is able to revert reserpine-induced oxidative stress and oral dyskinesia (3, 147). Finally, the treatment with 40 mg/kg vitamin E concomitant to the repeated treatment with 0.1 mg/kg reserpine (71, 167) prevented cognitive and motor impairments (168), as well as the reduction of TH immunostaining in rats (unpublished data).

These neurochemical imbalances resemble features of PD, as oxidative stress and DA depletion, which are keystones of the pathophysiology of the disease (33, 79). Thus, the pharmacological mechanism of reserpine comprises important qualities of PD pathophysiology and constitutes a good model for screening for candidate drugs to both symptomatic treatment and possible slowing of PD symptom progression. This advantage is reinforced by its low toxicity to researchers, low cost, and reproducibility among laboratories, which points out the reserpine model of PD as a suitable model for drug screening.

MOLECULAR AND NEUROCHEMICAL FEATURES OF THE RESERPINE MODEL

Despite the robust face and pharmacological validities, the current literature does not recognize reserpine as a useful PD model, arguing the lack of construct validity (61). This drawback is due to the experimental observations that (i) reserpine do not induce neurodegeneration and protein aggregation (61, 208); (ii) motor performance, monoamine content, and TH staining are partially restored after treatment interruption (144, 167); and (iii) reserpine lacks specificity regarding dopaminergic neurotransmission (10, 11, 119, 141, 144).

Nevertheless, the behavioral and neurochemical features of reserpine administration are highly reproducible with little

Table 1. Predictive validity of reserpine Parkinson's disease (PD) model effectiveness for symptomatic treatment of different motor disturbances in PD. The table was constructed and updated according to the table presented by Duty and Jenner (61). The drug list was compiled from the Parkinson's UK website: parkinsons.org.uk/content/drug-treatments-parkinsons (accessed 6 October 2014). Abbreviations: COMT = catechol-O-methyltransferase; DA = dopamine; MAO = monoamine oxidase.

Treatment	Rigidity	Hypokinesia	Catalepsy	Tremor	Oral dyskinesia	References
L-DOPA ± Carbidopa	+	+	+	+	–	(51, 85, 99, 133, 176)
DA agonists						
Bromocriptine	+	+	+	–	–	(98, 99, 133, 176)
Cabergoline	+	+	+	–	–	(133)
Pergoline	+	+	+	+	–	(51, 98, 122)
Pramipexole	–	+	+	–	–	(68, 122)
Ropinirole	–	–	+	–	–	(77)
Apomorphine	+	+	+	–	–	(85, 98, 99)
Glutamate antagonists						
Amantadine	+	+	–	+	–	(51, 85, 176)
Anticholinergics	–	–	–	–	–	–
Orphenadrine	–	–	–	–	–	–
Procyclidine	–	–	–	–	–	–
Trihexyphenidyl	+	–	–	–	–	(85)
Benztropine	+	–	–	–	–	(85)
COMT inhibitors						
Entacapone	–	–	–	–	–	–
Tolcapone	–	–	–	–	–	–
MAO-B inhibitors						
Rasagiline	–	+	–	–	–	(73)
Selegiline	+	+	–	–	+	(51, 176)
Antioxidative and Dietary therapy						
Vitamin E	–	–	–	–	+	(3, 66)
Co-enzyme Q10	–	–	–	–	–	–
Miscellaneous	–	–	–	–	+	(5, 24, 139, 147, 148)

variance across studies. Reserpine peripherally administered in the dose range of 1–10 mg/kg is known to produce a robust (70%–95%) depletion of monoamine content in several brain areas (10, 11, 58, 65, 86, 90, 119, 141, 144, 189; for a summary, see Table 2). This monoamine depletion starts 30 minutes after reserpine injection and may endure up to 14 days, finally returning to normal levels after 21 days of retrieval (90, 144). At first, the absence of specificity was considered a disadvantage regarding accurate modeling of PD neurochemistry. However, there is evidence of relevant alterations in 5-HT and NA imbalances in PD as well (28, 101, 194, 211, 213). This argues in favor of the resemblance of the neurochemical disruptions in the reserpine model with those in PD. Moreover, this characteristic is especially important to the aforementioned nonmotor deficits of PD. For instance, NA and 5-HT transmissions are related to cognitive and emotional function (130, 175). Accordingly, reserpine treatment results in monoamine depletion in areas involved in emotional processing—as the amygdala (119)—and cognition—as the hippocampus, cortex (9, 10), and prefrontal cortex (144). Furthermore, repeated reserpine treatment reduces TH staining in the hippocampus, prefrontal cortex, dorsal striatum, VTA, SN pars compacta (SNpc), and locus coeruleus (167).

Finally, acute or short-term DA depletion by reserpine treatment results in upregulation of D1, but not D2 (46, 132, 189). Nevertheless, long-term treatment also leads to D2 upregulation (140, 193). These neurochemical modifications also occur because of dopaminergic denervation in untreated PD patients. Functional

imaging techniques report upregulation of D2 receptor, whereas upregulation of D1 is not yet clearly defined (87, 95).

Another highly reproducible biochemical alteration in the reserpine model is the induction of oxidative stress. Reserpine, in the dose range of 1–10 mg/kg, is able to induce decreases in catalase, superoxide dismutase, total content of reduced glutathione, and ATP. Similarly, it increases glutathione peroxidase activity, oxidized glutathione, lipid peroxidation, nitric oxide (NO), and iron (2–4, 10, 11, 24, 35, 36, 65, 66, 71, 119, 138, 139, 147, 149, 159, 166, 174, 179, 186, 187; for a summary, see Table 3). Overall, there is an increase in oxidative damage. Nevertheless, some studies report contradicting results. Those differences seem to emerge from different dosage, treatment regimen, and brain area studied. For example, repeated treatment with low doses of reserpine (0.1 mg/kg) produced cumulative effects upon lipid peroxidation in the striatum, but not hippocampus, of rats (71). As well, catalase activity is generally reduced in all brain areas—except for the striatum in which some studies found increased activity (186, 187) or no significant differences (4, 66). This opposite outcome may be due to a differential fine-tuning of catalase activity regulation in the striatum, as catecholaminergic metabolism intrinsically leads to oxidative stress (127, 156). In fact, hydrogen peroxide (H₂O₂) is one of the main products of CA metabolism by MAO-A (127, 156), and naturally one may speculate that catalase in catecholaminergic neurotransmission is differentially modulated by increases in H₂O₂ in order to provide antioxidant protection. Indeed, this is endorsed by the observation

Table 2. Monoamine content depletion induced by different reserpine treatment regimens in rodents. Abbreviations: 5-HT = serotonin; BLA = basolateral amygdala; CTX = cortex; DA = dopamine; HPC = hippocampus; NA = noradrenaline; N/A = not applicable; PFC = prefrontal cortex; SN = substantia nigra; STR = striatum; THA = thalamus.

Dose (mg/kg)	Structure	Time window	DA	NA	5-HT	References
(50x) 0.01	STR	24 h	0%	~45%	0%	(141)
(50x) 0.1	STR	24 h	~90%	~90%	~65%	
(50x) 1.0	STR	24 h	~95%	~90%	~90%	
5.0	SN	2 h	~85%	N/A	N/A	(90)
		24 h	~70%			
	STR	2 h	>95%			
		24 h	>95%			
1.0	STR	6 h	~80%	N/A	~50%	Unpublished data
		24 h	~90%		~80%	
		96 h	~75%		~80%	
5.0	STR	24 h	~95%	N/A	N/A	(65)
5.0	STR	24 h	~70%	N/A	N/A	(189)
10.0	STR	18 h	~95%	N/A	N/A	(86)
	STR*	18 h	>95%			
1.0	STR	24 h	~55%	N/A	N/A	(58)
(3x) 1.0	BLA	24 h	~75%	~80%	~70%	(119)
(3x) 1.0	CTX	48 h	~75%	~60%	~70%	(10)
(3x) 1.0	CTX	48 h	~80%	~70%	~80%	(11)
	HPC	48 h	~70%	~60%	~85%	
3.0	THA*	24 h	~75%	>95%	>95%	(144)
	PFC*	24 h	~90%	>95%	~90%	

*Microdialysis studies.

Time window refers to time after last reserpine injection.

that catecholaminergic neurons are relatively abundant in populations of catalase-positive microperoxisomes (124). Thus, it seems that treatment duration and brain area studied define the extent of oxidative damage induced by reserpine.

The oxidative stress induced by reserpine is related to increased DA metabolism as a result of the reduction on the number of DA molecules in the vesicle (146) and increased DA turnover (67, 141, 179). Accordingly, MAO-A inhibitor reverts L-DOPA and reserpine induced increase in oxidized glutathione (179, 180). In addition, free DA and metabolites in the cytoplasm results in auto-oxidation of DA and DOPAC to their corresponding reactive quinones—DA-Q and DOPAC-Q, respectively—(12, 127, 156), which contributes to cell apoptosis and synuclein dimerization (84).

The generation of highly reactive molecules results in early cell damage—as consistently evidenced by lipid peroxidation (Table 3)—initiating proinflammatory signaling by tumor necrosis factor (TNF)- α and interleukin (IL)-1 β (10, 11). Subsequently, the increase in proinflammatory cytokines activates microglia, which leads to a vicious circle of adhesion, inflammation, and release of more cytokines. Activated microglia upon dopaminergic neurons also results in increased NO (10, 11, 24). Afterwards, NO—in the presence of superoxide (O₂⁻)—produces peroxynitrite (NO₃⁻) (127, 156), which is highly reactive and has been shown to inactivate TH via S-thiolation on cysteine residues (8, 96, 110, 111). In this context, repeated treatment with a low dose of reserpine (0.1 mg/kg) resulted in reduced TH immunostaining in several brain areas—that is hippocampus, prefrontal cortex, dorsal striatum, SNpc, and VTA (167).

Ultimately, these events may terminate in the commitment with apoptotic pathways. In other words, there is a reduction in anti-apoptotic molecules, as Bcl-2 (65, 119), and an increase in proapoptotic molecules, as caspase-3 (10, 11, 119).

Nevertheless, whether reserpine leads to permanent cell damage or neurodegeneration is not clear yet. In this respect, repeated treatment with 0.1 mg/kg of reserpine every other day for 20 days resulted in a reduction of TH immunostaining that was partially reversed after 30 days of treatment withdrawal (167). Likewise, the same protocol increased α -synuclein immunostaining in SN and dorsal striatum and these effects were reversed after treatment interruption (data not published). Of notice, such increase did not result in protein inclusions and studies addressing if actual neuronal loss occurs are currently being held. Thus, in light of the current evidence (extent of TH reduction and α -synuclein increase, restauration of motor performance, and reversion of reduction in TH and α -synuclein immunostaining after interruption of treatment), data regarding the repeated low-dose reserpine treatment should be interpreted in terms of TH expression reduction rather than neurodegeneration.

On the other hand, some evidence support long-lasting or permanent cellular and behavioral changes within a high dose chronic reserpine treatment. Treatment with 1 mg/kg of reserpine every other day for 6 weeks resulted in persistent behavioral and neurochemical changes (oral dyskinesia, DA depletion and D1 and D2 receptor upregulation) up to 60 days after treatment withdrawal (140). Thus, we do not discard the possibility of some extent of permanent cell damage or cell death after reserpine treatment, depending on dose and/or length of treatment.

In this context, untreated VMAT2 genetically deficient mice—which express only 5% of functional VMAT2—presents age-associated neurodegeneration in SNpc, locus coeruleus, and dorsal raphe, followed by α -synuclein accumulation and TH and tyramine transporter immunostaining reduction (41, 185). This VMAT2-deficient mice also presents L-DOPA responsive motor impairment, twofold increase in DA concentration in cytosol,

Table 3. Molecular changes related to oxidative stress induced by different reserpine treatment regimens in rodents. Abbreviations: CAT = catalase; GPX = glutathione peroxidase; GSH = reduced glutathione; GSSG = oxidized glutathione; GST = glutathione-S-transferase; LPO = lipid peroxide; NO = nitric oxide; NS = not significant; SOD = superoxide dismutase.

Structure	Dose (mg/kg)	Time window	CAT	SOD	GPX	GST	GSH	GSSG	GSSG/GSH	LPO	NO	References
Total brain	5.0	24 h				↓	↓			↑		(65)
	(3×) 1.0	3 h	↓	↓			↓			↑		(147)
	(3×) 1.0	24 h	↓	↓			↓			↑		(138)
	(3×) 1.0	24 h	↓	↓			↓			↑		(139)
	(3×) 1.0	17 days	↓	↓			↓			↑		(166)
Cortex	(2×) 1.0	24 h								NS		(149)
	(3×) 1.0	24 h								NS		(35)
	(3×) 1.0	48 h	↓	↓			↓			↑	↑	(11)
	(3×) 1.0	48 h	↓	↓						↑	↑	(10)
	(3×) 1.0	96 h								NS		(159)
Striatum	10	2 h						NS				(180)
	(10×) 0.1	24 h								↑		(2)
	(10×) 0.1	48 h								↑		(71)
	(2×) 0.5	24 h	NS									(66)
	(2×) 1.0	24 h	NS		NS							(4)
	(2×) 1.0	24 h	↑							↑		(187)
	(2×) 1.0	24 h							↑			(3)
	(2×) 1.0	24 h								↑		(36)
	(2×) 1.0	24 h	↑								↓	(186)
	(2×) 1.0	24 h								NS		(149)
	(3×) 1.0	24 h								↑		(35)
	(3×) 1.0	96 h								NS		(159)
Hippocampus	5.0	90 minutes			↑		NS	↑			↑	(24)
	10	2 h						↑				(180)
	(10×) 0.1	48 h								NS		(71)
	(2×) 1.0	24 h								NS		(149)
	(3×) 1.0	48 h	↓	↓						↑	↑	(11)
	(3×) 1.0	48 h	↓	↓						↑	↑	(10)
Substantia nigra	5.0	90 minutes			NS		NS	↑		↑	(24)	
Basolateral amygdala	(2×) 1.0	24 h							NS		(149)	
	(3×) 1.0	24 h	↓						↑		(119)	

Time window refers to time after last reserpine injection.

reduction in TH phosphorylation associated with catechol feedback, 95% of DA depletion, and increased DA turnover (50, 135, 185). Moreover, these alterations are accompanied by nonmotor impairments, such as deficit in olfactory discrimination, delayed gastric emptying, altered sleep latency, anxiety-like behavior, and age-dependent depressive behavior (185). In short, all behavioral and neurochemical alterations in VMAT2-deficient mice resemble the effects of reserpine treatment. As both reserpine and VMAT2-deficient mice models are similar in terms of functional construct, we speculate that neurodegeneration is a plausible outcome in long-term VMAT2 functional blockade by reserpine treatment. As mentioned earlier, this issue is currently under investigation.

In conclusion, reserpine treatment is able to induce (i) monoamine depletion, (ii) oxidative stress, (iii) inflammation, (iv) proapoptotic commitment, (v) reduction in tyrosine hydroxylase and increase in α -synuclein immunostaining, and (vi) DA receptors upregulation (for summary of neurochemical events after reserpine administration, see Figure 1). Despite that there is still no evidence of some important pathological features of PD—such as protein aggregation, permanent cellular damage, and

neurodegeneration—most of the reserpine-induced neurochemical alterations are clearly reminiscent of PD pathophysiology and thus holds a satisfactory resemblance to PD phenomenology. Therefore, the lack of construct validity should not be an argument against the use of the reserpine model to study PD.

It should be noted that the aforementioned toxin-based animal models do not account for all pathophysiological features of PD as well. 6-OHDA leads to neurodegeneration and motor impairment, but studies have not shown protein inclusions, while MPTP administration resulted in Lewy's body-like inclusions specifically in particular mice lineages. Likewise, rotenone treatment induces Lewy's body-like inclusions and neurodegeneration in rats, but the extent of neurodegeneration is highly variable (78, 81, 109, 113, 128, 190).

FINAL CONSIDERATIONS

In addition to the aforementioned features, one might question if the reserpine model mimics risk factors of PD, such as age and sex, for example. Neurochemical studies regarding age-related effects

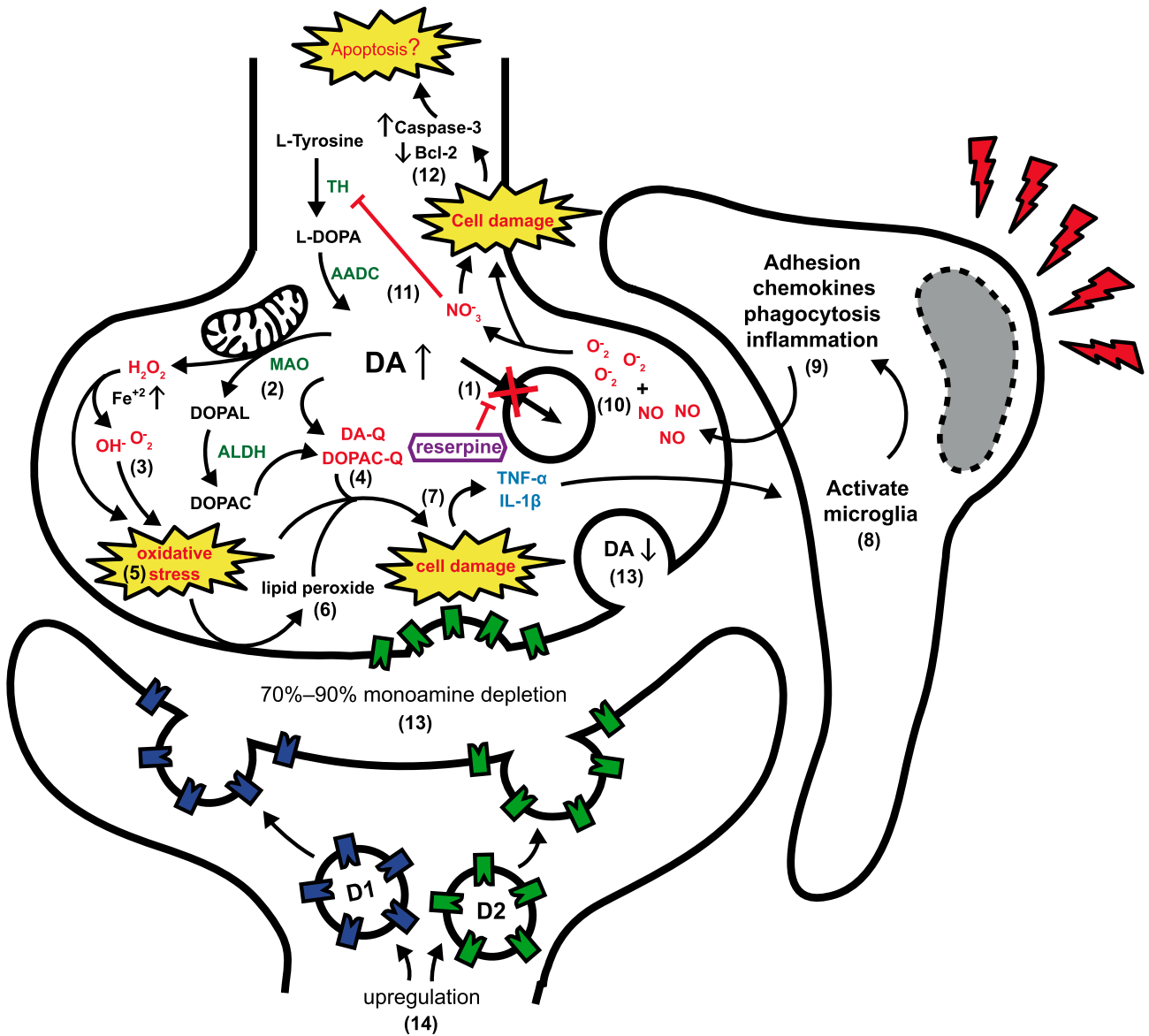


Figure 1. Neurochemical and molecular events after reserpine treatment. (1) Reserpine precludes dopamine (DA) storage. (2) Increased DA is metabolized in the cytoplasm (3) generating reactive oxygen species (ROS) and (4) highly reactive quinones (DA-Q and DOPAC-Q) (5) resulting in oxidative stress and (6) lipid peroxidation. (7) Accumulation of ROS and reactive quinones leads to cell damage and proinflammatory signalization. (8) Activation of microglia by tumor necrosis factor (TNF)-α and interleukin (IL)-1β (9) amplify proinflammatory signalization resulting

in (10) nitric oxide (NO) increase and peroxynitrite (NO₂) formation with free superoxide (O₂). (11) NO₃⁻ inhibits tyrosine hydroxylase (TH) activity and (12) reinforces cell damage committing cell fate in proapoptotic signalization. At the same time, (13) monoamine depletion in synaptic cleft results in (14) upregulation of D1 and D2 receptors on the postsynaptic and presynaptic membrane. AADC, aromatic L-amino acid decarboxylase; ALDH, aldehyde dehydrogenases; MAO, monoamine oxidase.

of reserpine treatment found that older rats presents reduced DA turnover (6) and a tendency to reduced DA recovery (153) compared with younger animals. Furthermore, oral dyskinesia is increased in older rats (2, 4, 35) and reserpine treatment results in cumulative (182) and persistent (21) oral dyskinesia in older animals. However, current literatures have not directly addressed the influence of age on other reserpine-induced motor deficits. Up to date, the low-dose repeated reserpine treatment has been conducted with 6-month-old rats (unlike studies with other

parkinsonism-inducing drugs, which are usually conducted with 3-month-old animals), but the studies did not include other age groups (71, 167).

Moreover, regarding sex differences, we have recently conducted the low-dose repeated reserpine treatment (0.1 mg/kg) in male and female Swiss mice and found that female mice took longer to develop motor impairment in the catalepsy (Figure 2A,B) and oral dyskinesia (Figure 2C) tests (refer to Figure 2 legend for methods and statistical analysis). Conversely,

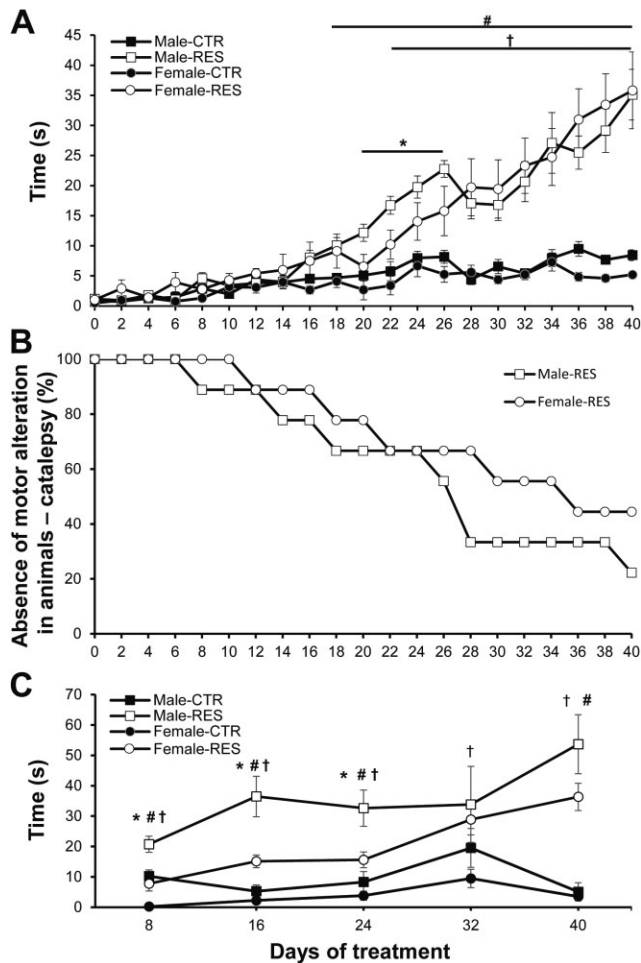


Figure 2. Motor deficits of repeated low-dose reserpine treatment in male and female mice. Male and female Swiss mice (6 months old; $n = 9$ per group) were repeatedly treated every other day with reserpine (0.1 mg/kg) (RES) or vehicle (CTR) for 40 days according to the protocol previously described for rats (71, 167). **(A)** Latency to step down in the catalepsy bar test. Mice were gently positioned with both forepaws in an elevated bar (6 cm). Catalepsy score was the mean of three measures of the latency to step down. Two-way analysis of variance (ANOVA) with repeated measures revealed effect of time [$f(20, 160) = 39.53$, $P < 0.001$], treatment [$f(3, 24) = 12.97$, $P < 0.001$], and time vs. treatment interaction [$f(60, 480) = 7.93$, $P < 0.001$]. **(B)** Percentage of male and female reserpine-treated mice without motor impairment (catalepsy test) across treatment. Animals were considered to present motor impairment when the catalepsy score was above the mean plus two standard errors of the mean of the respective CTR group. Gehan-Breslow-Wilcoxon test revealed that more female rats did not present motor deficit in the catalepsy bar test compared with male rats (chi-square = 4.065, $P = 0.043$). **(C)** Oral movement test. Mice were positioned in a small cage (20 × 25 × 20 cm) surrounded by mirrors and jaw-twitching time (s) was quantified within a 10-minute session by two blind observers. Two-way ANOVA with repeated measures revealed effect of time [$f(5, 40) = 15.86$, $P < 0.001$], treatment [$f(3, 24) = 25.58$, $P < 0.001$], and sex [$f(1, 8) = 42.07$, $P < 0.001$], as well as interactions for time vs. treatment [$f(15, 120) = 7.55$, $P < 0.001$], sex vs. time [$f(5, 40) = 18.35$, $P = 0.002$], and sex vs. treatment [$f(1, 24) = 37.93$, $P = 0.003$]. For all graphs, † $P < 0.05$ Female-RES vs. Female-CTR; # $P < 0.05$ male-RES vs. male-CTR; and * $P < 0.05$ male-RES vs. female-RES (Tukey's *post hoc* test for each day).

other study reported increased oral dyskinesia in female mice that was inconsistent at different time points (174). Contradicting results regarding oral dyskinesia might be explained by differences in protocol—that is length of treatment, dosage, and type of motor parameter (vacuous chewing vs. jaw twitching). Nevertheless, studies with CD-1 mice have suggested that female animals present a more efficient VMAT2 function (57, 58), which could explain the need of a longer treatment for female mice to develop the motor alterations (data displayed in Figure 2). Importantly, this result is in accordance with the lower incidence of PD in women (19, 62) and adds to the similarities between the reserpine model and the clinical condition.

The exposed prospect of reserpine-induced behavioral, pharmacological, and neurochemical effects restates the use of reserpine as a valuable and promising model for PD study. Thus, the current underuse of reserpine to investigate PD features should be reconsidered. Of notice, the use of reserpine could be important to the relevance of VMAT2 functionality to PD in humans. Indeed, polymorphisms in promoter regions that increases transcription of VMAT2 are protective against PD (31, 82) and reduction in VMAT2 and its mRNA in nigrostriatal neurons have been reported in PD patients (89, 131). Furthermore, VMAT2 is present in Lewy's bodies in the SN of PD patients (209) and VTA dopaminergic neurons that are spared in PD harbors higher levels of VMAT2 (131). Finally, increased cytoplasmic DA influences the conformational state of α -synuclein, promoting stabilization of its pathogenic form (75, 116). Thus, because functional VMAT2 expression is protective against dopaminergic neurodegeneration, its long-term blockage might represent an interesting approach to model PD.

In conclusion, we believe that the scientific effort on reserpine PD model validation should focus in answering whether neurodegeneration and cell death occur after chronic reserpine treatment, as well as the exploitation of the model to investigate progression of symptoms and neurochemical features of PD pathophysiology. We recently presented a low-dose reserpine-induced progressive model of PD that could be useful to investigate such inquiry (71, 167). Therefore, in view of the presented experimental evidence, the reserpine-induced PD model in rodents reaches robust face and pharmacological validity criteria, besides presenting a significant number of neurochemical and molecular features that closely resemble the pathophysiology of the disease. Taken together, these characteristics render the reserpine model a useful tool for PD basic research.

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