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Animal Models of Parkinson's Disease and Related Disorders

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INTRODUCTION

Animal models of neurological disorders are critical for determining underlying disease mechanisms and developing new therapeutic modalities. In general, the utility of an animal model for a particular disease is often dependent on how closely the model replicates all or part of the human condition. In Parkinson's disease (PD) and related parkinsonian disorders there now exists a variety of animal models, each of which makes a unique contribution to our understanding of the human condition.

These models have been derived in a variety of species (pig, nonhuman primate, rodent, and cat) using multiple techniques, including (1) surgical lesioning, (2) pharmacological manipulation, (3) administration of neuro-toxicants, and (4) genetic alterations. While these models are not identical to the human condition with respect to behavioral characteristics, brain anatomy, or disease progression, they have provided significant advancements in our understanding of the underlying mechanisms and treatment of movement disorders such as PD.

PD is characterized by bradykinesia, rigidity, postural instability, and resting tremor. The primary pathological and biochemical features of PD are the loss of nigrostriatal dopaminergic neurons in the substantia nigra, the appearance of intracellular inclusions called Lewy bodies, and the depletion of striatal dopamine. Clinical features are apparent when striatal dopamine depletion reaches 80% despite the fact that 45–60% of nigrostriatal dopaminergic neurons still remain (1). Since the destruction of the nigrostriatal system and consequent depletion of striatal dopamine are key features in the human condition, attempts have been made in animal models to disrupt an analogous anatomical area through surgical, pharmacological, or neurotoxicant manipulation.

The purpose of this chapter is to introduce the many different animal models utilized in PD research. Each model, when applicable, will be discussed with respect to its development, behavioral profile, biochemical and neuropathological alterations, and contribution to the field.

PHARMACOLOGICALLY INDUCED MODELS OF PARKINSON'S DISEASE

Reserpine

The first animal model for PD was demonstrated by Carlsson in the 1950s using rabbits treated with reserpine. Reserpine is a catecholamine-depleting agent that blocks vesicular storage of monoamines. The akinetic state, resulting from reserpine-induced dopamine depletion in the caudate and putamen, led Carlsson to speculate that PD was due to striatal dopamine depletion. This speculation was supported by the discovery of striatal dopamine depletion in postmortem brain tissue of PD patients and led to the subsequent use of levodopa (in conjunction with a peripheral dopa-decarboxylase inhibitor) for symptomatic treatment of PD (2,3). Thus, the initial observations derived from an animal model led to an important clinical therapy that remains a gold standard.

Alpha-Methyl-para-Tyrosine

Although less commonly used, alpha-methyl-para-tyrosine (AMPT), like reserpine, serves as an effective catecholamine-depleting agent (4). By directly inhibiting tyrosine hydroxylase (the rate-limiting enzyme in dopamine biosynthesis), the nascent synthesis of dopamine in neurons of the substantia nigra pars compacta and ventral tegmental area is prevented.

Both reserpine and AMPT have been used to discover new dopaminomimetics for the treatment of PD, but since their effects are

transient (hours to days), these models are primarily useful for acute studies. In addition, neither agent can duplicate the extensive biochemical and pathological changes seen in PD. Consequently, other models with long-lasting behavioral alterations have been sought using site-specific neurotoxicant injury.

NEUROTOXICANT-INDUCED MODELS OF PARKINSON'S DISEASE

6-Hydroxydopamine

6-Hydroxydopamine (6-OHDA or 2,4,5-trihydroxyphenylethylamine) is a specific catecholaminergic neurotoxin structurally analogous to both dopamine and noradrenaline. Acting as a "false-substrate," 6-OHDA is rapidly accumulated in catecholaminergic neurons. The mechanism of 6-OHDA toxicity is complex and involves (1) alkylation, (2) rapid auto-oxidization (leading to the generation of hydrogen peroxide, superoxide, and hydroxyl radicals), and (3) impairment of mitochondrial energy production (5,6). The 6-OHDA–induced rat model of PD was initially carried out by Ungerstedt in 1968, using stereotactic bilateral intracerebral injections into the substantia nigra or lateral hypothalamus (medial forebrain bundle) (7). The bilateral administration of 6-OHDA resulted in catalepsy, generalized inactivity, aphagia, and adipsia, and a high degree of animal morbidity and mortality. Consequently, the administration of 6-OHDA was modified to a unilateral intracerebral lesion (targeting the substantia nigra and/or medial forebrain bundle). With unilateral lesioning there was (1) minimal postoperative morbidity, (2) behavioral asymmetry, and (3) a nonlesioned side to serve as a control (8,9). An additional modification of 6-OHDA administration was chronic low-dose striatal injections. This led to progressive dopaminergic cell death that more closely resembled the human condition (10).

A distinctive behavioral feature of the unilateral lesioned model is rotation (11,12). This motor feature is due to asymmetry in dopaminergic neurotransmission between the lesioned and intact sides. Specifically, animals rotate away from the side of greater dopaminergic activity. Nomenclature describes the direction of rotation as either ipsilateral or contralateral to the lesioned side. Initial reports of rotation examined both spontaneous and pharmacologically manipulated rotation. Spontaneous rotation consists of ipsilateral rotation (towards the lesioned side), while pharmacologically induced rotation may be either contra- or ipsilateral rotation. For example, apomorphine and other dopamine agonists induce contralateral rotation (away from the lesioned side). This is due to their

direct action on supersensitized dopaminergic receptors on the lesioned side. Conversely, d-amphetamine phenylisopropylamine (AMPH) induces ipsilateral rotation by blocking dopamine reuptake and increasing dopamine receptor activity on the nonlesioned side. In general, a greater than 80% depletion of dopamine is necessary to manifest rotation in this model (4,13) Circling behavior can be measured either by observation or by special devices called rotometers. The rate of rotation correlates with the severity of the lesion, and animals with more extensive striatal dopamine depletions are less likely to show behavioral recovery. This simple model of rotation away from the side with the most dopamine receptor occupancy has recently proven much more complex and less predictable than previously thought, especially in the context of various pharmacological treatments and neuronal transplantation. In addition to rotation, other behavioral assessments in the 6-OHDA model may include tests of forelimb use, bilateral tactile stimulation, single limb akinesia, and bracing (for review see Ref. 14).

The 6-OHDA–lesioned rat model has proven to be a valuable tool in evaluating (1) the pharmacological action of new drugs on the dopaminergic system, (2) the mechanisms of motor complications, (3) the neuroplasticity of the basal ganglia in response to nigrostriatal injury, and (4) the safety and efficacy of neuronal transplantation in PD. Extensive pharmacological studies have utilized the 6-OHDA–lesioned rat to investigate the role of various dopamine receptor (D1–D5) agonists and antagonists and other neurotransmitter systems (including glutamate, adenosine, nicotine, or opiods) in modulating dopamine neurotransmission. These studies elucidate the role of these compounds in electrophysiological, behavioral, and molecular (signal transduction) properties of the basal ganglia. A review of the vast amount of pharmacological literature regarding this model is beyond the scope of this chapter (see Ref. 12).

The 6-OHDA–lesioned rat model has also been an important tool in elucidating the mechanism(s) underlying motor complications. The chronic administration of levodopa (over a period of weeks) to the 6-OHDA rat has been demonstrated to lead to a shortening response similar to the wearing-off complication in idiopathic PD (15). This altered motor response occurs when greater than 95% of nigrostriatal cells are lost. Studies using glutamate antagonists have demonstrated improvement in the wearing-off response and have implicated the role of glutamate receptor subtypes in the development of motor complications (16–18). These findings have been supported by molecular studies that demonstrate alterations in the phosphorylation state of glutamate receptor subunits of the NMDA subtype (19). Unlike the wearing-off phenomenon, 6-OHDA lesioned rats do not develop typical dyskinesias (20).

In the context of neuroplasticity, the 6-OHDA–lesioned rat model demonstrates behavioral recovery and has been instrumental in characterizing the neurochemical, molecular, and morphological alterations within the basal ganglia in response to nigrostriatal dopamine depletion (21). These mechanisms of neuroplasticity in surviving dopaminergic neurons and their striatal terminals include (1) increased turnover of dopamine and its metabolites, (2) alterations in the expression of tyrosine hydroxylase, the rate-limiting step in dopamine biosynthesis, (3) decreased dopamine uptake through altered dopamine transporter expression, (4) alterations in the electrophysiological phenotype (both pattern and rate of neuronal firing) of substantia nigra neurons, and (5) sprouting of new striatal dopaminergic terminals. These molecular mechanisms may provide new targets for novel therapeutic interventions such as growth factors to enhance the function of surviving dopaminergic neurons.

The 6-OHDA–lesioned rat model has also been useful for determining important parameters for successful transplantation. These parameters include (1) target site (striatum versus substantia nigra), (2) volume of innervation at the target site, (3) number of cells transplanted, (4) type and species of cells transplanted including fetal mesencephalon, engineered cell lines, and stem cells, (5) age of host and donor tissues, (6) pretreatment of transplant tissue or host with neurotrophic factors, antioxidants, immuno-suppressive therapy, or neuroprotective pharmacological agents, and (7) surgical techniques including needle design, cell suspension media, and transplant cell delivery methods (22,23). The near absence of dopaminergic neurons and terminals within the striatum due to 6-OHDA lesioning provide a template for the assessment of sprouting axons and terminals from the transplant. Measures of transplant success in this model include reduction in the rotational behavior and the survival, sprouting and innervation (synapse formation) of dopaminergic fibers within the denervated striatum. The reduction of rotational behavior suggests increased striatal dopamine production originating from the transplanted tissue. Interestingly, not all behavioral measures appear to respond to transplant. The advancements made in the 6-OHDA–lesioned rat provide a framework for the further testing of transplantation in nonhuman primates and future human clinical trials.

While the 6-OHDA–lesioned rat model has many advantages, it serves primarily as a model of dopamine dysfunction. Lesioning with 6-OHDA is highly specific for catecholaminergic neurons and does not replicate many of the behavioral, neurochemical, and pathological features of human PD. For example, the 6-OHDA–lesioned rat does not manifest alterations in the cholinergic and serotonergic neurotransmitter systems, which are commonly affected in PD. Stereotactic injections of 6-OHDA to precise targets does

not replicate the extensive pathology of PD where other anatomical regions of the brain (including the locus coeruleus, nucleus basalis of Meynert, and raphe nuclei) are affected. In addition, Lewy body formation, a pathological hallmark of PD, has not been reported in this model. Interestingly, a recent report using a regimen of chronic administration of 6-OHDA into the third ventricle did show a more extensive lesioning pattern reminiscent of human PD (24). In addition to the rat, other species including the nonhuman primate have served as models for 6-OHDA lesioning (25). Lesioning in nonhuman primates provides for the analysis of behaviors not observed in the rat, such as targeting and retrieval tasks of the arm and hand.

Overall, lesioning with 6-OHDA has provided a rich source of information regarding the consequences of precise dopamine depletion and its effects on rotational behavior, dopamine biosynthesis, biochemical and morphological aspects of recovery, and serves as an excellent template to study both pharmacological and transplantation treatment modalities for PD.

The Neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

The inadvertent self-administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by heroin addicts in the 1980s induced an acute form of parkinsonism, whose clinical and biochemical features were indistinguishable from idiopathic PD (26,27). Like PD, this MPTP cohort demonstrated an excellent response to levodopa and dopamine agonist treatment but developed motor complications within a short period of time (over weeks). The rapidity with which these motor complications appeared presumably reflected the severity of substantia nigra neuronal degeneration induced by MPTP. Given the above similarities between the human model of MPTP-induced parkinsonism and PD, it became evident that MPTP could be used to develop animal models of PD.

The subsequent administration of MPTP to a number of different animals has demonstrated a wide variety of sensitivity to the toxic effects of MPTP. These differences were shown to be species, strain, and age dependent. For example, the nonhuman primate is the most sensitive to the toxic effects of MPTP. The mouse, cat, dog, and guinea pig are less sensitive, and the rat is the least sensitive. Even within species there are strain differences. For example, the C57BL/6 mouse is the most sensitive of all mouse strains tested, while strains such as CD-1 appear almost resistant (28,29). Some differences amongst strains may also depend on the supplier; this may account for differences seen with the Swiss Webster mouse (30). In addition to strain, animal sensitivity to the neurotoxicant effects of MPTP may be influenced by the animal's age, with older mice, for example, being

more sensitive (31,32). Studies suggest that age-dependent differences may be due to differences in MPTP metabolism (33).

The mechanism of MPTP toxicity has been thoroughly investigated. The meperidine analog 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is converted to 1-methyl-4-pyridinium (MPP$^+$) by monoamine oxidase B. MPP$^+$ acts as a substrate of the dopamine transporter (DAT), leading to the inhibition of mitochondrial complex I, the depletion of adenosine triphosphate (ATP), and cell death of dopaminergic neurons. MPTP administration to mice and nonhuman primates selectively destroys dopaminergic neurons of the substantia nigra pars compacta (SNpc), the same neurons affected in PD (34). Similar to PD, other catecholaminergic neurons, such as those in the ventral tegmental area (VTA) and locus coeruleus, may be affected to a lesser degree. In addition, dopamine depletion occurs in both the putamen and caudate nucleus. The preferential lesioning of either the putamen or caudate nucleus may depend on animal species and regimen of MPTP administration (35–37). Unlike PD, Lewy bodies have not been reported, but eosinophilic inclusions (reminiscent of Lewy bodies) have been described in aged nonhuman primates (38). The time course of MPTP-induced neurodegeneration is rapid and therefore represents a major difference from idiopathic PD, which is a chronic progressive disease. Interestingly, data from humans exposed to MPTP indicate that the toxic effects of MPTP may be more protracted than initially believed (39). Details of MPTP toxicity and utility are described in Refs. 40 and 41.

The MPTP-Lesioned Mouse Model

The administration of MPTP to mice results in behavioral alterations that may resemble human parkinsonism. For example hypokinesia, bradykinesia, and akinesia can be observed through various behavioral analyses including open field activity monitoring, swim test, pole test, grip coordination, and rotorod. Whole body tremor and postural abnormalities have also been reported, but primarily in the acute phase (42). In general, these behavioral alterations tend to be highly variable, with some mice showing severe deficits while others show little or no behavioral change (for review see Ref. 42). This behavioral variability may be due to a number of factors, including the degree of lesioning, mouse strain, time course after lesioning, and the reliability and validity of the behavioral analysis.

The MPTP-lesioned mouse model has proven valuable to investigate potential mechanisms of neurotoxic induced dopaminergic cell death. For example, mechanisms under investigation have included mitochondrial dysfunction, energy (ATP) depletion, free-radical production, apoptosis, and glutamate excitotoxicity (41). In addition to its utility in studying acute

cell death, the MPTP-lesioned model also provides an opportunity to study injury-induced neuroplasticity. The MPTP-lesioned mouse displays the return of striatal dopamine several weeks to months after lesioning (35,37,43). The molecular mechanism of this neuroplasticity of the injured basal ganglia is an area of investigation in our laboratory and in others and appears to encompass both neurochemical and morphological components. In addition, work in our laboratory has shown that this plasticity may be facilitated through activity-dependent processes using treadmill training.

MPTP-Lesioned Nonhuman Primate

Administration of MPTP to nonhuman primates results in parkinsonian symptoms including bradykinesia, postural instability, and rigidity. In some species resting or action/postural tremor has been observed (44). Similar to PD, the MPTP-lesioned nonhuman primate responds to traditional antiparkinsonian therapies such as levodopa and dopamine receptor agonists. Following the administration of MPTP, the nonhuman primate progresses through acute (hours), subacute (days), and chronic (weeks) behavioral phases of toxicity that are due to the peripheral and central effects of MPTP. The acute phase is characterized by sedation, and a hyperadrenergic state, the subacute phase by the development of varying degrees of parkinsonian features, and the chronic phase by initial recovery (by some, but not all animals) followed by the stabilization of motor deficits (45). In general, the behavioral response to MPTP lesioning may vary at both the inter- and intraspecies levels. Variability may be due to age and species phylogeny. For example, older animals and Old World monkeys (such as rhesus *Macaca mulatta*, or African Green *Cercopithecus aethiops*) tend to be more sensitive than young and New World monkeys (such as the squirrel monkey, *Saimiri sciureus*, or marmoset, *Callithrix jacchus*) (46–48).

Behavioral recovery after MPTP-induced parkinsonism has been reported in most species of nonhuman primates. The degree and time course of behavioral recovery is dependent on age, species, and mode of MPTP administration (45). In general the more severely affected animal is less likely to recover (44). The molecular mechanisms underlying behavioral recovery of the nonhuman primate is a major focus of our laboratory. Results of our work and others have identified that the mechanisms underlying recovery may include (1) alterations in dopamine biosynthesis (increased tyrosine hydroxylase protein and mRNA expression) and turnover, (2) downregulation of dopamine transporter, (3) increased dopamine metabolism, (4) sprouting and branching of tyrosine hydroxylase fibers, (5) alterations of other neurotransmitter systems, including glutamate and serotonin, and (6) alterations of signal transduction pathways in both the direct (D1) and indirect (D2) pathways (49).

The administration of MPTP through a number of different dosing regimens has led to the development of several distinct models of parkinsonism in the nonhuman primate. Each model is characterized by unique behavioral and neurochemical parameters. As a result, numerous studies addressing a variety of hypotheses have been conducted. These studies consist of new pharmacological treatments, transplantation, mechanisms of motor complications, deep brain stimulation, behavioral recovery, cognitive impairment, and the development of novel neuroprotective and restorative therapies. For example, in some models there is profound striatal dopamine depletion and denervation with little or no dopaminergic axons or terminals remaining. This model provides an optimal setting to test fetal tissue grafting since the presence of any tyrosine hydroxylase positive axons or sprouting cells would be due to transplanted tissue survival. Other models have less extensive dopamine depletion and only partial denervation with a modest to moderate degree of dopaminergic axons and terminals remaining. This partially denervated model best resembles mild to moderately affected PD patients. Therefore, sufficient dopaminergic neurons and axons as well as compensatory mechanisms are likely to be present. The effects of growth factors (inducing sprouting) or neuroprotective factors (promoting cell survival) are best evaluated in this situation. The following section reviews the most commonly used MPTP-lesioned nonhuman primate models.

In the *systemic lesioned* model, MPTP may be administered via intramuscular, intravenous, intraperitoneal, or subcutaneous injection (50–53). This leads to bilateral depletion of striatal dopamine and nigrostriatal cell death. A feature of this model is that the degree of lesioning can be titrated, resulting in a range (mild to severe) of parkinsonian symptoms. The presence of clinical asymmetry is common, with one side more severely affected. Levodopa administration leads to the reversal of all behavioral signs of parkinsonism in a dose-dependent fashion. After several days to weeks of levodopa administration, animals develop reproducible motor complications, both wearing-off and dyskinesia. Animal behavior in this model and others may be assessed using (1) cage-side or video-based observation, (2) automated activity measurements in the cage through infrared based motion detectors or accelerometers, and (3) examination of hand-reaching movement tasks. The principal advantage of this model is that the behavioral syndrome closely resembles the clinical features of idiopathic PD. The systemic model has partial dopaminergic denervation bilaterally and probably best represents the degree of loss seen in all stages of PD, including end-stage disease where some dopaminergic neurons are still present. This model is well suited for therapeutics that interact with remaining dopaminergic neurons, including growth factors, neuroprotective

agents, and dopamine modulation. The easily reproducible dyskinesia in this model allows for extensive investigation of its underlying mechanism and treatment. Disadvantages of this model include spontaneous recovery in mildly affected animals. Alternatively, bilaterally severely affected animals may require extensive veterinary care and dopamine supplementation.

Administration of MPTP via unilateral intracarotid infusion has been used to induce a hemiparkinsonian state in the primate, called the *hemi-lesioned* model (54). The rapid metabolism of MPTP to MPP$^+$ in the brain may account for the localized toxicity to the hemisphere ipsilateral to the infusion. Motor impairments appear primarily on the contralateral side. Hemi-neglect, manifested by a delayed motor reaction time, also develops on the contralateral side. In addition, spontaneous ipsilateral rotation may develop. Levodopa administration reverses the parkinsonian symptoms and induces contralateral rotation. Substantia nigra neurodegeneration and striatal dopamine depletion (>99%) on the ipsilateral side to the injection is more extensive than seen in the systemic model. The degree of unilateral lesioning in this model is dose dependent.

Major advantages of the *hemi-lesioned* model include (1) the ability for animals to feed and maintain themselves without supportive care, (2) the availability of the unaffected limb on the ipsilateral side to serve as a control, and (3) the utility of the dopamine-induced rotation for pharmacological testing. In addition, due to the absence of dopaminergic innervation in the striatum, *the hemi-lesioned* model is well suited for examining neuronal sprouting of transplanted tissue. A disadvantage of this model is that only a subset of parkinsonian features is evident, which are restricted to one side of the body, a situation never seen in advanced PD.

The *bilateral intracarotid* model employs an intracarotid injection of MPTP followed several months later by another intracarotid injection on the opposite side (55). This model combines the less debilitating features of the carotid model as well as creating bilateral clinical features, a situation more closely resembling idiopathic PD. The advantage of this model is its prolonged stability and limited inter-animal variability. Similar to the hemi-lesioned model, where there is extensive striatal dopamine depletion and denervation, the bilateral intracarotid model is well suited for evaluation of transplanted tissue. However, levodopa administration may result in only partial improvement of parkinsonian motor features and food retrieval tasks. This can be a disadvantage since high doses of test drug may be needed to demonstrate efficacy, increasing the risk for medication related adverse effects.

A novel approach to MPTP lesioning is the administration of MPTP via intracarotid infusion followed by a systemic injection. This *overlesioned* model is characterized by severe dopamine depletion ipsilateral to the

MPTP-carotid infusion and a partial depletion on the contralateral side due to the systemic MPTP injection. Consequently, animals are still able to maintain themselves due to a relatively intact side. The behavioral deficits consist of asymmetrical parkinsonian features. The more severely affected side is contralateral to the intracarotid injection (56). Levodopa produces a dose-dependent improvement in behavioral features, but the complications of levodopa therapy such as dyskinesia have not been as consistently observed. This model combines some of the advantages of both the systemic and intracarotid MPTP models, including stability. This model is suitable for both transplant studies, utilizing the more depleted side, and neuroregeneration with growth factors, utilizing the partially depleted side where dopaminergic neurons still remain.

Finally, the *chronic low-dose* model consists of intravenous injections of a low dose of MPTP administration over a 5- to 13-month period (57). This model is characterized by cognitive deficits consistent with frontal lobe dysfunction reminiscent of PD or normal aged monkeys. These animals have impaired attention and short-term memory processes and perform poorly in tasks of delayed response or delayed alternation. Since gross parkinsonian motor symptoms are essentially absent, at least in early stages, this model is well adapted for studying cognitive deficits analogous to those that accompany idiopathic PD.

The MPTP-lesioned nonhuman primate has provided a valuable tool for investigating potential mechanisms underlying motor complications related to long-term levodopa use in human idiopathic PD. The MPTP-lesioned nonhuman primate has been shown to demonstrate both wearing-off and dyskinesia. Although the etiology of dyskinesia is unknown, electrophysiological, neurochemical, molecular, and neuroimaging studies in the nonhuman primate models suggest that the pulsatile delivery of levodopa may lead to (1) changes in the neuronal firing rate and pattern of the globus pallidus and subthalamic nucleus, (2) enhancement of D1- and/or D2-receptor–mediated signal transduction pathways, (3) super-sensitivity of the D2 receptor; (4) alterations in the phosphorylation state and subcellular localization of glutamate (NMDA subtype) receptors, (5) modifications in the functional links between dopamine receptor subtypes (D1 and D2, and D1 and D3), (6) changes in glutamate receptors (AMPA and NMDA receptor subtypes), and (7) enhancement of opiod-peptide–mediated neurotransmission (58–62).

While the presence of a nigral lesion has long been considered an important prerequisite for the development of dyskinesia in the MPTP model, recent studies demonstrate that even normal nonhuman primates when given sufficiently large doses of levodopa (with a peripheral decarboxylase inhibitor) over 2–8 weeks may develop peak-dose dyskinesia

(63). The high levels of plasma levodopa in this dosing regimen may serve to exhaust the buffering capacity within the striatum of the normal animal and therefore lead to pulsatile delivery of levodopa and priming of postsynaptic dopaminergic sites for dyskinesia.

In addition to its central effects, the administration of MPTP may lead to systemic effects, which may prove detrimental to any animal during the induction of a parkinsonian state. For example, the peripheral conversion of MPTP to MPP^+ in the liver could lead to toxic injury of the liver and heart. To address these potential peripheral effects of MPTP, squirrel monkeys were administered MPTP (a series of 6 subcutaneous injections of 2 mg/kg, free-base, 2 weeks apart) and were given a comprehensive exam 1, 4, and 10 days after each injection. This exam included measurements of body weight, core body temperature, heart rate, blood pressure, liver and kidney function, and white blood cell count. Biochemical markers of hepatocellular toxicity were evident within days of MPTP lesioning and persisted for several weeks after the last injection. In addition, animals had significant hypothermia within 48 hours after lesioning that persisted for up to 10 days after the last MPTP injection. The pathophysiology of these effects may be directly related to MPTP itself and/or its metabolites. The systemic effects of MPTP on animal models should be taken into consideration during the design of any pharmacological study.

Methamphetamine

Amphetamine and its derivatives (including methamphetamine) lead to long-lasting depletion of both dopamine and serotonin when administered to rodents and nonhuman primates (64,65). Methamphetamine (METH), one of the most potent of these derivatives, leads to terminal degeneration of dopaminergic neurons in the caudate-putamen, nucleus accumbens, and neocortex. In contrast to MPTP, the axonal trunks and soma of SNpc and VTA neurons are spared (66). However, there have been occasional reports of METH-induced cell death in the substantia nigra (67). In general, the effects of severe METH lesioning are long-lasting. There is evidence of recovery of dopaminergic innervation depending on the METH regimen and species used (68). Despite the severe depletion of striatal dopamine, the motor behavioral alterations seen in rodents and nonhuman primates are subtle (69).

The neurotoxic effects of METH are dependent on the efflux of dopamine since agents that deplete dopamine or block its uptake are neuroprotective (70,71). The metabolic mechanisms underlying METH-induced neurotoxicity involve the perturbation of antioxidant enzymes such as glutathione peroxidase or catalase, leading to the formation of reactive

oxygen/nitrogen species including H_2O_2, superoxide, and hydroxyl radicals (72–76). The administration of antioxidant therapies or overexpression of superoxide dismutase (SOD) in transgenic mice models is neuroprotective against METH toxicity (77,78). In addition, both glutamate receptors and nitric oxide synthase (NOS) are important to METH-induced neurotoxicity since the administration of either NMDA receptor antagonists or NOS inhibitors are also neuroprotective (79). Other factors important to METH-induced neurotoxicity include the inhibition of both tyrosine hydroxylase and dopamine transporter activity and METH-induced hyperthermia (75).

The administration of METH to adult animals has played an important role in testing the molecular and biochemical mechanisms underlying dopaminergic and serotonergic neuronal axonal degeneration, especially the role of free radicals and glutamate neurotransmission. Understanding these mechanisms has led to testing different neuroprotective therapeutic modalities. An advantage of the METH model over MPTP is that the serotonergic and dopaminergic systems can be lesioned in utero during the early stages of the development of these neurotransmitter systems. Such studies have indicated that there is a tremendous degree of architectural rearrangement that occurs within the dopaminergic and serotonergic systems of injured animals as they develop. These changes may lead to altered behavior in the adult animal (80).

In light of the toxic nature of these compounds in animals, studies in humans have suggested that abusers of METH and substituted amphetamines (including MDMA, "ecstasy") may suffer from the long-lasting effects of these drugs (81,82). Specifically, these individuals may be prone to develop parkinsonism (83).

Rotenone

Epidemiological studies have suggested that environmental factors such as pesticides may increase the risk for PD (84). The demonstration of specific neurochemical and pathological damage to dopaminergic neurons by the application of various pesticides such as rotenone (an inhibitor of mitochondrial complex I) have supported these epidemiological findings. For example, using a chronic rotenone infusion paradigm, Greenamyre and colleagues reported degeneration of a subset of nigrostriatal dopaminergic neurons, the formation of cytoplasmic inclusions, and the development of parkinsonian behavioral features (including hunched posture, rigidity, unsteady movement, and paw tremor) in the rat (85). Studies examining the effects of various pesticide applications in animal models may lead to insights into the mechanisms of neuronal death in PD (86).

GENETIC MODELS OF PARKINSON'S DISEASE

In addition to pharmacological and neurotoxicant models of PD, there are spontaneous rodent (such as the *weaver* mouse and *AS/AGU* rat) and transgenic mouse (including parkin, α-synuclein) models that provide important avenues to investigate the basal ganglia.

Spontaneous Rodent Models for Parkinson's Disease

There are several naturally occurring spontaneous mutations in rodents that are of particular interest in PD. Spontaneous rodent models include the *weaver, lurcher, reeler, Tshr*^hyt, *tottering, coloboma* mice and the *AS/AGU* and *circling* (*ci*) rat. These models possess unique characteristics that may provide insight into neurodegenerative processes of PD and related disorders. Several of these spontaneous rodent models display altered dopaminergic function or neurodegeneration and have deficits in motor behavior (87). For example, the *weaver* mouse displays cell death of dopaminergic neurons while the *tottering* mouse displays tyrosine hydroxylase hyperinnervation. The *AS/AGU* rat is a spontaneous model characterized by progressive rigidity, staggering gait, tremor, and difficulty in initiating movements (88). Microdialysis in the *AS/AGU* rat model has revealed that even prior to dopaminergic neuronal cell death, there is dysfunction in dopaminergic neurotransmission that correlates with behavioral deficits. Another potentially interesting rodent model is the *circling* (*ci*) rat (89). This animal model displays spontaneous rotational behavior as a result of an imbalance in dopaminergic neurotransmission despite the absence of asymmetrical nigral cell death.

Transgenic Mouse Models

The development of transgenic animal models is dependent on identifying genes of interest. A transgenic mouse is an animal in which a specific gene of interest has been altered through one of several techniques including: (1) the excision of the host gene (knock-out), (2) the introduction of a mutant gene (knock-in), and (3) the alteration of gene expression (knock-down). In PD, one source of transgenic targeting is derived from genes identified through epidemiological and linkage analysis studies. α-Synuclein and parkin are examples of genes that have been identified through linkage analysis. Other transgenic animals have been developed based on the identification of genes important for normal basal ganglia and dopaminergic function. These transgenic mouse lines target several genes, including superoxide dismutase (SOD), glutathione reductase, monoamine oxidase (MAO), dopamine receptors (D1, D2), dopamine transporter (DAT), caspases, neurotrophic

factors (BDNF and GDNF), and neurotransmitter receptors (NMDA and AMPA). Once the transgene has been constructed, the degree of its expression and its impact on the phenotype of the animal depends on many factors, including the selection of sequence (mutant versus wild-type), site of integration, number of copies recombined, selection of transcription promoter, and upstream controlling elements (enhancers). Other important factors may include the background strain and age of the animal. These different features may account for some of the biochemical and pathological variations observed among transgenic mouse lines. Two examples of recent transgenic mouse lines are discussed.

Parkin

An autosomal recessive form of juvenile parkinsonism (AR-JP) led to the identification of a gene on chromosome 6q27 called parkin (90,91). Mutations in parkin may account for the majority of autosomal recessive familial cases of PD. Parkin protein has a large N-terminal ubiquitin-like domain and C-terminal cysteine ring structure and is expressed in the brain (92–94). Recent biochemical studies indicate that Parkin protein may play a critical role in mediating interactions with a number of different proteins involved in the proteasome-mediated degradation pathway, including α-synuclein (95,96). Mutations of the parkin gene have been introduced into transgenic mice. At present there is very little known about pathological or behavioral alterations due to mutations in Parkin protein. However, parkin transgenic models enable investigation of the ubiquitin-mediated protein degradation pathways and their relationship to neurodegenerative disease.

α-Synuclein

Rare cases of autosomal dominant familial forms of PD (the Contursi and German kindreds) have been linked to point mutations in the gene encoding α-synuclein (97). The normal function of α-synuclein is unknown, but its localization and developmental expression suggests a role in neuroplasticity (98,99). The disruption of normal neuronal function may lead to the loss of synaptic maintenance and subsequent degeneration. It is interesting that mice with knockout of α-synuclein are viable, suggesting that a "gain-in-function" phenotype or other protein-protein interactions may contribute to neurodegeneration. Although no mutant forms of α-synuclein have been identified in idiopathic PD, its localization to Lewy bodies (including PD and related disorders) has suggested a patho-physiological link between α-synuclein aggregation and neurodegenerative disease. To investigate these potential mechanisms, several groups have developed transgenic mouse models. An interesting caveat is that the mutant allele of α-synuclein in the Contursi kindred is identical to the wild-type mouse, suggesting that protein

expression and/or protein-protein interactions may be more important than loss of function due to missense mutation. Therefore, transgenic mouse models developed for α-synuclein focus on altered protein expression through the use of different promoters and gene cassette constructs. Some transgenic mouse lines show pathological changes in dopaminergic neurons (including inclusions, decreased striatal dopamine, and loss of striatal tyrosine hydroxylase immunoreactivity), behavioral deficits (rotorod and attenuation of dopamine-dependent locomotor response to amphetamine), while other lines show no deficits (100–102). No group has reported the loss of substantia nigra dopaminergic neurons. This range of results with different α-synuclein constructs from different laboratories underscores the important link between protein expression (mutant vs. wild-type alleles) and pathological and behavioral outcome. Important applications of α-synuclein transgenic mice are occurring at the level of understanding the role of this protein in basal ganglia function. For example, the response of α-synuclein expression to neurotoxic injury as well as interactions with other proteins, including parkin, will provide valuable insights into mechanisms important to neurodegeneration (95).

Invertebrate Models

Recent developments of invertebrate transgenic models (such as in *Drosophila melanogaster*) for α-synuclein, parkin, and other genes of interest provide another avenue to investigate the function of proteins of interest in PD. In addition, the application of dopaminergic-specific toxins, such as 6-OHDA to *Caenorhabditis elegans*, may provide another tool for understanding mechanisms of cell death (103). Unlike mammalian animal models, invertebrate models tend to be less expensive and greater numbers can be generated in shorter periods of time. These advantages offer a means for high-volume screening of pharmacological agents for the treatment of PD (104,105).

MODELS OF PD VARIANTS

While the models discussed in the above sections provide insights into PD as well as related disorders such as multisystem atrophy (MSA) and progressive supranuclear palsy (PSP), other models have been developed that share a greater similarity with these variants.

Multisystem Atrophy and Striatonigral Degeneration

Multisystem atrophy (MSA) is a variant of PD characterized by a combination of clinical symptoms involving cerebellar, extrapyramidal,

and autonomic systems. The predominant subtype of MSA is striatonigral degeneration (SND), a form of levodopa-unresponsive parkinsonism. Neuropathological changes of SND include degeneration of the nigrostriatal pathway, medium spiny striatal GABAergic projection pathways (putamen greater than caudate), as well as other regions of the brain stem, cerebellum, and spinal cord. Inclusion-like aggregates that immuno-stain for ubiquitin and α-synuclein are seen in oligodendrocytes and neurons.

The basis for developing an animal model for SND emerged from established animal models for both parkinsonian (having SNpc pathology) and Huntington's disease (HD) (having striatal pathology). For example, rodent models for SND have been generated through sequential stereotactic injections of 6-OHDA and quinolinic acid (QA) into the medial forebrain bundle and striatum, respectively, or striatal injections of MPP^+ and 3-nitropropionic acid (3-NP)(106–108). These double-lesioning models are characterized morphologically by neuronal degeneration in the SNpc and ipsilateral striatum. The order of neurotoxic lesioning may influence the degree of nigral or striatal pathology. For example, animals receiving 6-OHDA prior to QA exhibit predominantly nigral pathology, while animals receiving QA prior to 6-OHDA show predominantly striatal pathology. This may be due to QA-induced terminal damage or other complex interactions after lesioning that reduce terminal uptake of 6-OHDA. Glial inclusions have not been reported in any of these models indicating a significant difference compared with the human condition.

Motor deficits in models for MSA and SND are assessed by ipsilateral and contralateral motor tasks (including stepping response, impaired paw reaching, and balance) and drug-induced circling behavior. As described earlier, characteristic drug-induced circling behavior occurs after 6-OHDA lesioning resulting in ipsilateral rotation in response to amphetamine and contralateral rotation in response to apomorphine. The subsequent striatal lesioning with QA diminishes (or has no effect on) amphetamine-induced ipsilateral rotation and reduces (or abolishes) apomorphine-induced contralateral rotation. This observation may be mediated by dopamine release on the intact side (in response to amphetamine) and/or the loss of dopamine receptor activation on the lesioned side (in response to apomorphine). The lack of response to apomorphine has been shown to correlate with the volume of the striatal lesion and is analogous to the diminished efficacy of levodopa therapy observed in the majority of SND patients.

A nonhuman primate (*Macaca fasicularis*) model of SND has been generated through the sequential systemic administration of MPTP and 3-NP (106,109). The parkinsonian features after MPTP lesioning are levodopa responsive, but subsequent administration of 3-NP worsens motor

symptoms and nearly eliminates the levodopa response. Levodopa occasionally induces facial dyskinesia as sometimes seen in human MSA. Similar to SND morphological changes include cell loss in the SNpc (typical of MPTP-lesioning) and severe circumscribed degeneration of striatal GABAergic projection neurons (typical of 3-NP lesioning). Despite the similarities with the human condition, the MSA model is characterized by an equal degree of lesioning in the putamen and caudate nucleus, while in human SND the putamen is more affected. In addition, inclusion bodies that may underlie the pathogenesis of SND have not been reported in the nonhuman primate model.

The Tauopathies Including Progressive Supranuclear Palsy and Other Tau-Related Disorders

The low molecular weight microtubule-associated protein tau has been implicated in a number of neurodegenerative diseases, including Alzheimer's disease, progressive supranuclear palsy (PSP), Pick's disease, frontotemporal dementia with parkinsonism (FTDP), and amyotrophic lateral sclerosis/parkinsonism-dementia complex (ALS/PDC) of Guam. Together these neurodegenerative diseases comprise what is referred to as tauopathies, since they share common neuropathological features including abnormal hyperphosphorylation and filamentous accumulation of aggregated tau proteins. Reports in the literature have implicated either alternative RNA splicing (generating different isoforms) or missense mutations as mechanisms underlying many of the tauopathies. Therefore, transgenic mice have been generated that overexpress specific splice variants or missense mutations of tau (110). One such transgenic line has been developed to overexpress the shortest human tau isoform (111). These mice showed progressive motor weakness, intraneuronal and intra-axonal inclusions (detectable by 1-month postnatal), and reduced axonal transport. Fibrillary tau inclusions developed in the neocortical neurons after 18 months of age implicating age-specific processes in the pathogenesis of fibrous tau inclusions. An interesting tau transgenic line has been developed in *Drosophila melanogaster*, where expression of a tau missense mutation showed no evidence of large filamentous aggregates (neurofibrillary tangles). However, aged flies showed evidence of vacuolization and degeneration of cortical neurons (112). These observations suggest that tau-mediated neurodegeneration is age-dependent and may take place independent of protein aggregation.

CONCLUSIONS

Our understanding of Parkinson's disease and related disorders has been advanced through animal models using surgical, pharmacological, and neurotoxicant manipulation. The nonhuman primate, rodent, cat, and pig models have contributed to the development of symptomatic (dopamine modulation), neuroprotective (antioxidants, free-radical scavengers), and restorative (growth factors, transplantation) therapies. In addition, these animal models have furthered our understanding of motor complications (wearing off and dyskinesia), neuronal cell death, and neuroplasticity of the basal ganglia. Future direction in PD research is through the continued development of animal models with altered genes and proteins of interest. In conjunction with existing models, these genetic-based models may lead to the eventual cure of PD and related disorders.

ACKNOWLEDGMENTS

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