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# Animal models of Parkinson's disease: an empirical comparison with the phenomenology of the disease in man

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**Summary.** Animal models are an important aid in experimental medical science because they enable one to study the pathogenetic mechanisms and the therapeutic principles of treating the functional disturbances (symptoms) of human diseases. Once the causative mechanism is understood, animal models are also helpful in the development of therapeutic approaches exploiting this understanding. On the basis of experimental and clinical findings. Parkinson's disease (PD) became the first neurological disease to be treated palliatively by neurotransmitter replacement therapy.

The pathological hallmark of PD is a specific degeneration of nigral and other pigmented brainstem nuclei, with a characteristic inclusion, the Lewy body, in remaining nerve cells. There is now a lot of evidence that degeneration of the dopaminergic nigral neurones and the resulting striatal dopamine-deficiency syndrome are responsible for its classic motor symptoms akinesia and bradykinesia. PD is one of many human diseases which do not appear to have spontaneously arisen in animals. The characteristic features of the disease can however be more or less faithfully imitated in animals through the administration of various neurotoxic agents and drugs disturbing the dopaminergic neurotransmission.

The cause of chronic nigral cell death in PD and the underlying mechanisms remain elusive. The partial elucidation of the processes underlie the selective action of neurotoxic substances such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has however revealed possible molecular mechanisms that give rise to neuronal death. Accordingly, hypotheses concerning the mechanisms of these neurotoxines have been related to the pathogenesis of nigral cell death in PD.

The present contribution starts out by describing some of the clinical, pathological and neurochemical phenomena of PD. The currently most important animal models (e.g. the reserpine model, neuroleptic-induced

catalepsy, tremor models, experimentally-induced degeneration of nigrostriatal dopaminergic neurons with 6-OHDA, methamphetamine, MPTP, $MPP^+$, tetrahydroisoquinolines, β-carbolines, and iron) critically reviewed next, and are compared with the characteristic features of the disease in man.

**Keywords:** Parkinson's disease, animal model, MPTP, 6-hydroxydopamine, methamphetamine, iron, oxidative stress, calcium, pathogenesis, neurotoxins, neurodegeneration, TaClo, tetrahydroisoquinolines, β-carbolines, catalepsy.

## Introduction

Animal models are an important aid in experimental medical science because they enable one to study the pathogenetic mechanisms and the therapeutic principles of treating the functional disturbances (symptoms) of human diseases. Once the causative mechanism is understood, animal models are also helpful in the development of neuroprotective approaches exploiting this understanding. Animal models are only valuable as models for human diseases to the extent that they faithfully reflect the diseased state in man: ideally they should exactly simulate the pathological, histological and biochemical changes of the disease and their resulting functional disturbances.

Parkinson's disease (PD) is one of many human diseases which do not appear to have spontaneously arisen in animals. The characteristic features of the disease can however be more or less faithfully imitated in animals through the administration of various neurotoxic agents such as 6-hydroxydopamine (6-OHDA), methamphetamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and iron. The motor symptoms of PD and the underlying pathological changes are observed not only in laboratory animals such as mice, rats and non-human primates, but also in domestic animals like the horse. It was recently reported (Wang et al., 1991) that horses which had eaten the yellow starthistle *centaurea solstitialis L.* with their feed, after two or three months developed symptoms such as rigid facial muscles, irrational chewing movements, spasms of the tongue and diminished feed-intake, followed later on by hypokinesia, a general loss of reaction to stimuli and ultimately death. Neuropathological investigations of the brains of these animals showed bilateral necrosis of the globus pallidus and the substantia nigra (SN), and on the basis of these findings the syndrome was classified as nigro-pallidal encephelomalacia.

The present contribution starts out by describing some of the characteristic clinic, neuropathological, and neurochemical phenomena of PD. The currently most important animal models are described next, and are then compared with the characteristic features of the disease in man.

## The phenomenology of the Parkinson syndrome

### *1. Clinical features*

In 1817 James Parkinson in his "Essay on the Shaking Palsy" first described the disease which came to be known as PD or idiopathic Parkinson syndrome.

On the basis of etiological factors one can distinguish the frequently encountered idiopathic form from the various less frequently occurring symptomatic forms and from those disease presentations that are accompanied by multisystem degeneration. With the exception of the drug-induced syndrome, all forms of PD are chronically progressive.

The fully-developed Parkinson syndrome is a particularly characteristic disease state. It is defined by the triad of symptoms comprising akinesia, rigidity and tremor, which are not always equally strongly manifested. To some degree tremor predominates without motor functions showing the typical akinetic confinement. In other cases symptoms of akinesia and rigidity are the ruling symptoms. These cardinal symptoms may be accompanied by postural anomalies, vegetative symptoms and psycho-organic disturbances such as depression, slowness of affect, and occasionally dementia (Birkmayer and Riederer, 1985). The vegetative disturbances include increased salivation, seborrhoea, constipation, hot flushes and hot sweats as well as circulatory disturbances (Birkmayer and Riederer, 1985).

### *2. Neuropathology*

The main morphological changes in PD, which has been most frequently found in 60–75% of all autopsies carried out on patients with clinically diagnosed PD, is damage to the SN pars compacta, which is already apparent macroscopically by a depigmentation above all of the ventro-lateral portion (Jellinger, 1988; Gibb et al., 1990). This is possibly due to degeneration of dopaminergic, neuromelanin-containing neurons. Microscopic immunohistochemical analysis does in fact show that in precisely this region of the brain of Parkinsonian patients there is a definite (60–85%) loss of neuromelanin-containing tyrosine hydroxylase (TH)-immunoreactive neurones (TH is the enzyme which catalyses the rate-limiting step of catecholamine biosynthesis) (for a review see Jellinger, 1991). However, other pigmented nuclei of the brainstem are also affected, such as the locus ceruleus or the dorsal vagal nucleus, with some variability in both degree of severity and precise topical location (Jellinger, 1991). Severe lesions occur in the central amygdaloid nucleus, in nuclei projecting to the cerebral cortex in a non-specific manner, and in nuclei regulating endocrine and autonomic functions (Braak et al., 1995). Beyond this, one also finds damage in regions of the brain which contain neither neuromelanin nor catecholaminergic neurons. For example, in patients with PD who have suffered from dementia, autopsies show that the nucleus basalis Meynert shows a 60–77% loss of cholinergic neurons in comparison to age-matched individuals without neurologically or psychologically apparent symptoms (Jellinger, 1991).

The second characteristic pathological change is considered to be the appearance of Lewy bodies (Gibb, 1989). Lewy bodies, which to some extent may also be found in the brains of older people, are characteristic cytoplasmic inclusions which exhibit both halo and nucleus. In 85–100% of autopsies on patients with clinically diagnosed PD these Lewy bodies can be demonstrated in catecholaminergic neurons of the SN; however, in many cases they

also occur in other brain regions, such as for example the cortex, the magnocellular basal forebrain nuclei, and even the spinal cord (Gibb, 1989; Braak et al., 1995).

## *3. Pathobiochemistry of PD*

### 3.1 Changes in dopaminergic systems

Because of the degeneration of the dopaminergic nigro-striatal neurons, one finds a drastic reduction in the dopamine levels of the striatum (Table 1) and other nuclei of the basal ganglia. This finding, first described by Ehringer and Hornykiewicz (1960) was subsequently confirmed by countless investigations (for review see Gerlach and Riederer, 1993). The deficiency in dopamine, which is most pronounced in the putamen, is characteristic of all forms of PD, and is not apparent in other neurodegenerative diseases such as for example in Huntington's chorea (Reynolds and Garrett, 1986).

The morphologically-demonstrated loss of neurons in the pars compacta of the SN correlates significantly with the reduction of dopamine in the

**Table 1.** Pathobiochemistry of the dopaminergic nigro-striatal system in Parkinson's disease

| | Brain region | % of normal values | Reference* |
|---|---|---|---|
| *Concentrations of dopamine and of its metabolites* | | | |
| Dopamine | Substantia nigra | 17 | Birkmayer and Riederer (1975) |
| | Caudate nucleus | 10 | |
| | Putamen | 4 | |
| DOPAC | Substantia nigra | 2 | Riederer et al. (1986) |
| | Putamen | 10 | |
| HVA | Substantia nigra | 48 | Riederer et al. (1986) |
| | Putamen | 29 | |
| *Activity of dopamine-metabolising enzymes* | | | |
| Tyrosine hydroxylase | Substantia nigra | 46 | Riederer et al. (1978) |
| | Caudate nucleus | 60 | Rausch et al. (1988) |
| | Putamen | 16 | Riederer et al. (1978) |
| DOPA decarboxylase | Caudate nucleus | 9 | Lloyd and Hornykiewicz (1970) |
| | Putamen | 4 | |
| Catechol O-methyl-transferase | Substantia nigra | 82 | Lloyd et al. (1975) |
| | Caudate nucleus | 70 | |
| | Putamen | 78 | |
| Monoamine oxidase-B | Substantia nigra | 125 | Riederer et al. (1989a) |
| *Dopamine uptake sites* | | | |
| [$^3$H]Mazindol uptake | Caudate nucleus | 32 | Mizukawa et al. (1993) |
| | Putamen | 16 | |

*DOPA* 3,4-dihydroxyphenylalanine, *DOPAC* 3,4-dihydroxyphenylacetic acid, *HVA* homovanillic acid. *For further reading and original data see references indicated in Table 1

striatum, and the extent of the dopamine deficit correlates with the degree of akinesia (Bernheimer et al., 1973). Furthermore, the analysis of clinical and biochemical correlations show that the characteristic symptoms of the Parkinson syndrome only begin to appear when over 70% of the originally present dopamine content has been lost (Bernheimer et al., 1973; Riederer and Wuketich, 1976). This dopamine deficit in the striatum forms the rationale for the dopamine-substitution therapy using L-DOPA (3,4-dihydroxyphenylalanine, also called levodopa), which even today is the basic therapy for PD.

In addition to the drastic reduction of striatal dopamine concentrations, one also finds strongly diminished amounts of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), reduced activity of the dopamine-synthesising enzymes TH and DOPA decarboxylase, and a reduction in the number of dopamine uptake sites (Table 1). On the other hand there are no – or only slight – reductions in the activities of the dopamine-deactivating enzymes catechol-O-methyltransferase (COMT) and monoamine oxidase-B (MAO-B), which are predominantly localised extraneuronally or in the glia. These findings point to the destruction of the pre-synaptic dopaminergic neurons and to an overall decreased dopaminergic neurotransmission in the basal ganglia of patients with PD. The losses in the content of dopamine metabolites as well as in the activity of the enzyme activities are however less pronounced than those in dopamine content (Table 1). The way that this is interpreted is that the still intact dopaminergic neurons have to release more dopamine in order to maintain adequate functional dopaminergic neurotransmission.

In contrast to this pre-synaptic disturbance, the post-synaptic dopamine receptors in the striatum appear to be unaltered and fully functional. The evidence for this is comes on the one hand from receptor-binding studies which have overwhelmingly demonstrated unaltetred receptor densities in patients with PD (for reviews see Gerlach and Riederer, 1993) and on the other hand also the clinical experiences with dopamine-receptor agonists in the treatment of patients with PD.

The reduction in dopamine levels does not only occur in the basal ganglia, however, but also to a variable degree in the mesocortical projections (gyrus cinguli, hippocampus, frontal and entorhinal cortexes) and mesolimbic projections (hypothalamus, nucleus accumbens, corpus amygdaloideum, area olfactoria) of the ventral tegmental area (VTA) (for reviews see Gerlach and Riederer, 1993). Although no morphological-neurochemical correlations are available at present, one can nevertheless conclude that these dopamine deficiencies are a consequence of the destruction of dopaminergic neurons in the VTA: losses amounting to 45–60% of the cells in this region are quoted in the literature (Jellinger, 1988). Reduced activites of TH (Riederer et al., 1978) and diminished dopamine levels have also been demonstrated in adrenal tissue (Carmichael et al., 1988) and in the retina (Harnois and Diapolo, 1990) of patients with PD. These findings all point to a general involvement of the dopaminergic system in this disease.

### 3.2 Changes in other neurotransmitter and neuromodulator systems

In the late phases of the disease these changes in the dopaminergic systems are however also accompanied by changes in other neurotransmitter and neuromodulator systems. The regional differential reduction in noradrenaline concentrations is similarly connected with a destruction of noradrenergic neurons and has been held responsible for certain non-motor symptoms of the disease (dementia, depression, vegetative side-effects; for a review see Gerlach et al., 1994). The fall in serotonin concentration that has been demonstrated in all regions of the brain that have been investigated, although there is up to now no evidence for any degenerative process, is referred to a variable involvement of the raphe nuclei (Jellinger, 1988). Parkinsonian patients suffering from dementia additionally have a degeneration of the cholinergic nucleus basalis Meynert cortical projection (Jellinger, 1988). The lowering of the activity of glutamate decarboxylase, the enzyme responsible for the biosynthesis of GABA (γ-aminobutyric acid), is possibly also a consequence of the primary degeneration of dopaminergic nigro-striatal neurons (see Gerlach and Riederer, 1993). A whole range of neuropeptides (leucine- and methionine-enkephalins, substance P, cholecystokinin) are to various degrees reduced in concentration in various regions of the nigro-striatal system and in the VTA. Somatostatin is however only reduced specifically in the frontal cortex and in the hippocampus (see Gerlach and Riederer, 1993).

### 3.3 Biochemical changes which indirectly suggest a pathological mechanism

Table 2 summarizes some of the neurochemical changes providing indirect evidence of "oxidative stress" as a cause of neurodegeneration in PD. The "oxidative stress" hypothesis infers an imbalance between the formation of cellular oxidants and the antioxidative processes. "Oxidative stress", due to the excessive formation of hydrogen peroxide and oxygen-derived free radicals such as hydroxyl radicals ($^{\cdot}OH$), superoxide radicals ($^{\cdot}O_2$) or nitric oxide (NO) can cause cell damage through chain reactions of membrane lipid peroxidation and/or alterations in membrane fluidity (Halliwell, 1992). Hydrogen peroxide is produced in human tissues by several enzymes, such as superoxide dismutase (SOD), L-amino acid oxidase, glycollate oxidase, xanthine oxidase, and MAO. In dopaminergic nerve cells it is mainly generated by MAO via deamination of dopamine, and non-enzymatically by autoxidation of dopamine. Hydrogen peroxide is relatively inert and not toxic to cells. However, damage is done when hydrogen peroxide interacts with the reduced forms of transitional metal ions [e.g. iron (II) or copper (I)] and decomposes to the highly reactive hydroxyl free radical (the Fenton reaction). In addition, hydroxyl radicals are produced in the mitochondria of nerve cells during oxidative phosphorylation.

In the brains of patients who have died with PD, specifically the SN has been found to contain diminished activities of glutathione peroxidase and catalase (hydrogen peroxide metabolizing enzymes), as well as diminished concentrations of reduced glutathione (GSH) (Table 2; for a review see Olanow, 1993; Gerlach et al., 1996b). These findings suggest an aberrant

**Table 2.** Pathobiochemical findings in Parkinson's disease providing indirect evidence of "oxidative stress"

| | Brain region | % of normal values | Reference* |
|---|---|---|---|
| *1. Disturbed iron metabolism* | | | |
| Ferritin concentration | Substantia nigra | 129 (s.) | Riederer et al. (1989b) |
| | Putamen | 137 (n.s.) | |
| Total iron concentration | Substantia nigra | 177 (s.) | Riederer et al. (1989b) |
| | Putamen | 81 (n.s.) | |
| | Globus pallidus | 120 (n.s.) | |
| | Cortex | 100 | |
| Iron(II)/Iron(III) ratio | Substantia nigra | 43 (s.) | Sofic et al. (1988) |
| | Putamen | 77 (n.s.) | |
| | Globus pallidus | 80 (n.s.) | |
| | Cortex | 122 (n.s.) | |
| *2. Inactivation of hydrogen peroxide-metabolising enzymes* | | | |
| GSH concentration | Substantia nigra | 53 (s.) | Sofic et al. (1992) |
| Glutathione peroxidase activity | Substantia nigra | 80 (s.) | Kish et al. (1985) |
| Catalase activity | Substantia nigra | 64 (s.) | Ambani et al. (1975) |
| | Putamen | 67 (s.) | |
| *3. Indications of decreased free radical detoxification* | | | |
| Superoxid dismutase activity | Substantia nigra | 133 (s.) | Saggu et al. (1989) |
| | Cerebellum | 95 (n.s.) | |
| *4. Indirect indications of free radical-induced damage* | | | |
| Polyunsaturated fat concentration | Substantia nigra | 85 (s.) | Dexter et al. (1989) |
| | Putamen | 94 (n.s.) | |
| | Caudate nucleus | 95 (n.s.) | |
| | Globus pallidus | 94 (n.s.) | |
| | Cortex | 104 (n.s.) | |
| Basal concentrations of thiobarbituric acid-reactive substances | Substantia nigra | 135 (s.) | Dexter et al. (1989) |
| | Putamen | 105 (n.s.) | |
| | Caudate nucleus | 111 (n.s.) | |
| | Globus pallidus | 111 (n.s.) | |
| | Cortex | 94 (n.s.) | |
| 8-Hydroxy-2′deoxy-guanosine | Substantia nigra | 238 (s.) | Sanchez-Ramos et al. (1994) |
| | Putamen | 250 (s.) | |
| | Caudate nucleus | 275 (s.) | |
| | Globus pallidus | 168 (n.s.) | |
| | Cortex | 260 (n.s.) | |
| | Cerebellum | 110 (n.s.) | |
| | Hippocampus | 111 (n.s.) | |

*GSH* reduced glutathione, *n.s.* no significant difference from normal values, *s.* significant different from normal values. *For further reading and original data see references indicated in Table 2

metabolism of hydrogen peroxide. There is also a site-specific increase of SOD activity in the SN as well as a slightly increased MAO-B activity (Table 1). Both of these findings point to an increased hydrogen peroxide formation in the SN of patients with PD. The increase of iron in the SN with a shift of the nigral iron(II)/iron(III) ratio from 2:1 in control brains to 1:2 in the brains of patients with PD, indicates an increased rate of synthesis of hydroxyl radicals. Necropsy studies have further shown increased basal levels of thiobarbituric acid-reactive substances in the SN of patients with PD (a measure of secondary products of lipid peroxidation) coupled with a decrease in the levels of polyunsaturated fatty acids (the substrates for lipid peroxidation). In addition, there seems to be radical-induced DNA damage in the nigro-striatal system as indicated by raised 8-hydroxy-2′deoxyguanosine (Table 2), a product of free-radical attack on guanine in DNA.

Of particular interest in this connection is the observation that patients with PD had a lowered NADH-dehydrogenase (complex I) activity (for a review see Reichmann et al., 1993; Schapira, 1994). Inhibition of complex I by paraquat, the classical inhibitor of this enzyme complex, likewise leads to formation of superoxide free radicals (Turrens and Boveris, 1980). A recently published investigation showed that chronic L-DOPA therapy results in this altered activity of the cerebral respiratory chain enzyme (Przedborski et al., 1993): not only dopamine but also L-DOPA, its metabolic precursor, lead to reversible inhibition of complex I activity, and this inhibition can be lifted by GSH, vitamin C, SOD and catalase, but also by MAO-B inhibitors. From these results it may be concluded that in patients with PD the observed reduction in the activity of complex I is probably caused by an increased "oxidative stress" which in turn is provoked by an increased dopamine turnover.

## Animal experimental models of the Parkinson syndrome

### *1. Pharmacologically-induced functional disturbances of dopamine neurotransmission*

#### 1.1 The reserpine model

The starting point for the discovery that a depletion of dopamine from the striatum is responsible for the motor symptoms of PD, was the observation by Carlsson et al. (1957) that an akinetic state could be elicited in rats by systemic administration of reserpine, and that this state could be alleviated by L-DOPA. In the rat, reserpine induces a reduction in motor activity (akinesia, hypokinesia, catalepsy), in addition to a tremor which has, as yet, not been explored pharmacologically (Haefely, 1978). The precise pharmacological mechanisms underlying reserpine's effects are still not completely understood. However, it has been established that, at high doses, intraneuronal storage vesicles are affected via magnesium- and ATP-dependent mechanisms, and depleted of their stores of dopamine and other neurotransmitters, such as adrenaline, noradrenaline, histamine and serotonin. Further, post-synaptic re-uptake at least in the short-term no longer occurs (McGeer et al., 1987).

In the sixties and seventies, reserpine-induced inhibition of motor activity in rodents was widely used for investigating symptomatic anti-Parkinson treatments (for a review see Haefely, 1978). In addition to L-DOPA, amphetamine and its derivatives, dopamine-receptor agonists (Haefely, 1978) and NMDA (NMDA is N-methyl-D-aspartate. and its receptor is a subtype of the glutamate receptor) antagonists such as MK-801, amantadine and memantine (Danysz et al., 1994) were found to be effective. However, because reserpine induces the release of a variety of neurotransmitters, this approach is now rarely used. A modified form is used by Carlsson's group (see for example Carlsson and Carlsson, 1989) as a model of akinesia to investigate symptomatic treatment strategies. The laboratory animals used in this model are white NMRI mice, who are injected, 18 hours prior to assessment of motor activity, with 10mg/kg reserpine intraperitoneally (i.p.), and, to potentiate the effect, two hours prior with α-methyl-p-tyrosine (500mg/kg) which inhibits dopamine synthesis. Motor activity is evaluated with an electronic measuring device which measures the mean activity of the animals in a given time-period by means of light barriers.

### 1.2 Neuroleptic-induced catalepsy

Classic neuroleptics such as haloperidol, as dopamine-D2-receptor antagonists, give rise to effects which are similar to those which occur after reserpine-treatment (for reviews see Colpaert, 1987; Sanberg et al., 1988). In rats, catalepsy, defined as the delayed or absent correction of an abnormal positioning of the extremeties (Fog, 1972), may be simply assessed. An example of the many variants of this kind of catalepsy test is the "crossed extremities" test described by Boissier and Simon (1963), in which the experimenter crosses the animal's extremities on the same side: the animal is judged to be cataleptic if it is unable to correct this unnatural positioning within ten seconds. The substances with anticataleptic activity – in decreasing order of effectiveness – are anticholinergic drugs, dopamine-releasing drugs, dopamine agonists, and L-DOPA (Haefely, 1978). Recent pharmacological investigations have shown that the catalepsy produced by haloperidol (0.5mg/kg i.p.) can also be antagonised by NMDA-receptor antagonists: Amantadine and MK-801 produced dose-dependent inhibition of haloperidol-induced catalepsy, while memantine was less efficacious at 10mg/kg but, due to myorelaxant activity, did not have an anticataleptic effect at 20mg/kg (Danysz et al., 1994).

In-depth studies of haloperidol- and morphine-induced cataleptic states has led to the conclusion that catalepsy represents a complex state of behaviour inadequately described by classical behavioural tests (De Ryck et al., 1980). More recently, "catalepsy" describes a behavioural state which has been caused by an experimentally-induced dopamine depletion, and which consists of motor-inhibition (akinesia), muscular rigidity (rigor) and tremor of the extremities (Copaert, 1987; Sanberg et al., 1988). In this state the neuronal systems of the brain which are involved in the voluntary initiation of motor programmes are functionally inactive, in contrast, brain systems involved in the reflex control of posture and in the maintenance of equilibrium are func-

tioning normally (Schallert and Teitelbaum, 1981). Although the execution of motor programmes in cataleptic animals is disturbed, strong external stimuli such as pinching the tail, cold water or loud noises can activate release mechanisms and stimulate the cataleptic animal into activity (for a review see Schmidt et al., 1992).

#### 1.3 Haloperidol-induced akinesia and bradykinesia

The administration of haloperidol to rats at low doses (0.15 or 0.3 mg/kg i.p.) may also result in a behavioural state resembling the symptoms of akinesia and bradykinesia in PD (for a review see Schmidt et al., 1992). To measure the influence of dopamine in the control and initiation of motor activity in rats, Hauber (1990) has developed a technique in which a rapid initiation of motor activity, in response to a stimulus, results in a food reward. The experimental conditions consist of a starting-box, a track and a target box. A trained rat is placed in the starting-box facing a closed trap-door that prevents access to the track. After variable delay periods, a stimulus signals the opening of the starting-gate. The rat responds to the stimulus by starting to move, and to run along the track until it reaches the target box to be rewarded with food. The latent period between the signal to start and the beginning of locomotor activity is measured by a photoelectrical switch in front of the starting-box and a stimulus platform which is mounted underneath the starting-box. The following parameters can be measured: reaction time (the interval between presentation of the stimulus and the beginning of locomotion); the movement time (the interval between the beginning of movement and exit from the starting box); and the initial acceleration (which is the resultant of the quantitation of the development of motor force and the signal amplitude of motor acceleration).

The systemic administration of haloperidol at the indicated dosage elicits a specific deterioration in motor behaviour, such as delayed initiation of motion measured as a increase in reaction time (described as akinesia) and a slowed execution of the movement (described as bradykinesia), measured as extended movement time and diminished initial acceleration (Hauber, 1990).

### *2. Experimentally-induced degeneration of nigro-striatal dopaminergic neurons*

#### 2.1 The 6-hydroxydopamine model

##### 2.1.1 Neuropathological and neurochemical changes

The neurotoxic action of 6-OHDA was first observed during investigations of the autonomic nervous system: in this system 6-OHDA leads to a noradrenaline-depletion of several months' duration (Porter et al., 1963) and to a selective destruction of noradrenergic nerve-endings (Thoenen and Tanzer, 1968). Systemically administered 6-OHDA is unable to cross the blood-brain barrier. However, direct application of small doses to the lateral ventricle (150 μg free base) or to various brain structures (8 μg free base) leads to a selective destruction of catecholaminergic neurons (Ungerstedt,

1968; Bloom et al., 1969; Uretsky and Iversen, 1970). This results in a depletion of dopamine, noradrenaline and adrenaline in the affected brain regions; while concentrations of other neurotransmitters (acetylcholine, serotonin, GABA) are found to be unchanged (for a review see Zigmond and Stricker, 1989). In addition, further biochemical and histological changes which such as reduced levels of catecholamine metabolites and tetrahydrobiopterin (the co-factor of TH), and a diminished number of catecholamine re-uptake sites and TH-immunoreactive neurons, also point to a destruction of catecholaminergic neurons. Detailed histological investigations confirm the specificity of the neurotoxic effect of 6-OHDA on catecholaminergic neurons (for a review see Zigmond and Stricker, 1989). The simultaneous application of inhibitors of the high-affinity noradrenaline transport system (e.g. desipramine) can even improve the specificity to the extent that the damage can be entirely restricted to dopaminergic neurons (Breese and Traylor, 1970).

Although these findings with 6-OHDA-produced lesions have been described in rats, these effects may also be seen in other rodent species (mice) and other non-rodent species (cats, dogs and monkeys) (for a review see Zigmond and Stricker, 1989).

#### 2.1.2 Behavioural changes after bilateral lesions

Bilateral stereotactic injections of 6-OHDA into rat striatum lead to a massive mortality of dopaminergic nerve cells in the SN and to a corresponding depletion of dopamine in the striatum. Animals become akinetic, aphagic and adipsic and mortality is high (Ungerstedt, 1968). Less severe forms of this akinetic-aphagic syndrome may be elicited in rats by stereotactic injection of 6-OHDA into the median forebrain bundle (anteriolateral hypothalamus), which produces a marked depletion of noradrenaline from the hypothalamus and of dopamine from the striatum (Smith and Young, 1974). This hypokinesia may be abolished by L-DOPA and various dopamine-receptor agonists but not by anticholinergic substances (Butterwort et al., 1978). However recently, because of the intensive nature of animal care required, bilateral 6-OHDA application is rarely used.

#### 2.1.3 Behavioural changes after unilateral lesions

Unilateral stereotactic injection of 6-OHDA into the striatum (Andén et al., 1966; Ungerstedt and Arbuthnott, 1970) or the SN (During et al., 1992) of the rat leads to massive death of the dopaminergic nerve cells on the same side and to a corresponding depletion of dopamine in the striatum. The rat with this unilateral lesion of the nigrostriatal dopaminergic neurons constitutes an interesting experimental model, and one still in use. Animals with such a lesion show asymmetric motor behaviour for a short time immediately after the intervention, but subsequently behave normally, exhibiting asymmetric motor behaviour only following severe psychic stress. Following the systemic administration of dopamine-receptor agonists, L-DOPA and dopamine-releasing drugs, a distinct asymmetry appears in the longitudinal axis of the body and a characteristic circling behaviour is initiated, which can be quanti-

tated in a rotation-box (Hefti et al., 1980; During et al., 1992): L-DOPA and direct dopamine-receptor agonists such as bromocriptine lead to contralateral rotation (towards the undamaged side); while dopamine releasing substances, such as amphetamine and amantadine, lead to ipsilateral rotation (in the direction of the damaged side). For example, i.p. injection of 1 mg/kg of apomorphine to a rat with a 90% striatal dopamine loss elicits more than 20 contralateral turns per five minute period (Hefti et al., 1980).

This characteristic rotational behaviour is explained in the following way:

1. The degeneration of the nigro-striatal dopaminergic neurons leads to supersensitivity of post-synaptic dopamine receptors in the striatum. Thus receptors on the lesioned side are more sensitive to dopamine-receptor agonists than those on the intact side.
2. Dopamine-releasing drugs can stimulate the post-synaptic receptors in the striatum of the undamaged side, but not those on the damaged side.
3. Efferent nerve fibres from the striatum inhibit the ipsilateral motor activity. This inhibitory influence of the striatum is diminished by activation of the dopamine receptors in the striatum; thus stimulation of the dopamine receptors increases motor activity. If one applies a direct dopamine receptor agonist, then the reaction of the supersensitive dopamine receptors leads to a stronger motor activation on the lesioned compared to the intact side, with the result that the stimulation on the lesioned side is dominant resulting in turns towards the undamaged (contralateral) direction. On the other hand, predominantly dopamine-releasing drugs overwhelmingly stimulate the dopamine receptors on the undamaged side resulting in turns towards the lesioned (ipsilateral) side.

This rotational model clearly distinguishes drugs with predominantly dopamine receptor agonist activity from those with predominantly dopamine-releasing activity: However, substances in which both these activities are combined cannot be distinguished. More recent investigations have demonstrated that the contralateral rotation with apomorphine can be potentiated by systemic application of glutamate receptor agonists such as 6-nitro-sulphamoyl-benzo-quinoxalinedione (NBQX, an antagonist of the AMPA-receptor) or 3-carboxy-piperazine-propylphosphonic acid (CPP, a competitive inhibitor of the NMDA-receptor); but this requires only minimal doses of L-DOPA (Wachtel et al., 1992). Systemic application of the non-competitive NMDA-antagonist MK-801 on the other hand induces ipsilateral rotation (Goto et al., 1993). This result cannot be plausibly explained on the basis of the explanations indicated above: possibly some pharmacodynamic processes are responsible. Dopamine which is released by the glutamate-receptor antagonist appears insufficient on its own to stimulate the supersensitive dopamine receptors in the lesioned striatum.

#### 2.1.4 The mechanism of the neurotoxic action

Because of its structural similarity to other catecholamines, 6-OHDA is apparently selectively taken up into the appropriate neurons by the high-affinity

catecholamine transport system. This assumption would explain the specificity of its neurotoxic action towards catecholaminergic neurons. In fact, marked, long-lasting (10 month) decrease in the number of TH-immunoreactive neurons in the ipsilateral SN following unilateral injection of 6-OHDA into the striatum of rats was demonstrated (Ichitani et al., 1994).

6-OHDA is thought to induce nigro-striatal dopaminergic lesions via generation of hydrogen peroxide and the hydroxyl radicals derived from it (Heikkila and Cohen, 1972; Sachs and Jonsson, 1975), presumably initiated by a transition metal such as iron. In fact, it has been shown by magnetic resonance (MR) imaging (Hall et al., 1992), and by neurochemical and histochemical studies (e.g. Oestreicher et al., 1994) that iron is increased in the striatum of 6-OHDA-lesioned rats. Furthermore, 6-OHDA releases iron from ferritin in vitro (Monteiro and Winterbourn, 1989). The decisive part played by iron in the formation of hydroxyl free radicals from hydrogen peroxide is also evident in the fact that intranigral injection of iron(III) produces similar neurotoxic effects to those produced by 6-OHDA (Ben-Shachar and Youdim, 1991; Sengstock et al., 1992).

Indirect evidence that hydroxyl radicals are involved in the neurotoxic effects of 6-OHDA have been provided by investigations which demonstrated the influence of this neurotoxin on radical-detoxifying systems (Perumal et al., 1992). Thus 6-OHDA does not only lead to a reduction of GSH in the affected brain regions (22% in the striatum, for example), but also to a loss of SOD-activity (22% in the striatum). Furthermore, malondialdehyde level and the level of conjugated dienes were increased by 43% and 40%, respectively, in the striatum following 6-OHDA treatment (Kumar et al., 1995). The decrease in SOD-activity is explained on the basis of oxygen free radicals (Hodgson and Fridovich, 1975). Interestingly, it was recently shown (Glinka and Youdim, 1995) that 6-OHDA is even more toxic to complex I than the 1-methyl-4-pyridinium ion ($MPP^+$, the probable neurotoxic form of MPTP). The inhibition of complex I by $MPP^+$ leads to the mitochondrial production of superoxide free radicals (Hasegawa et al., 1990; Cleeter et al., 1992), hydrogen peroxide and hydroxyl radicals (Adams et al., 1993). Inhibition of complex I by paraquat also leads to the formation of superoxide radicals (Turrens and Boveris, 1980). The partial, or even complete, prevention of the neurotoxic effects of 6-OHDA and iron by prior administration of iron chelating agents (Ben-Shachar et al., 1991), vitamin E (Cadet et al., 1989; Perumal et al., 1992) and the MAO-B inhibitor selegiline (Knoll, 1986) may also be regarded as indirect evidence for the formation of free radicals.

### 2.2 The methamphetamine model

The amphetamines are psychostimulatory drugs with addictive potential. Their activity is primarily associated by their dopamine-releasing mechanism (Seiden et al., 1975; McMillen, 1983). In very high doses these indirectly acting psychostimulants do however also have a neurotoxic activity in rodents (rats, mice and guineapigs) and non-human primates (Seiden et al., 1975; Wagner et al., 1979, 1980).

2.2.1 Neuropathological, neurobiochemical and behavioural changes

A single dose or multiple applications of methamphetamine to rats or mice leads to a reduction of the dopamine, DOPAC and HVA concentrations in the striatum (Table 3). This reduction seems to be restricted to the nigrostriatal dopaminergic system as dopamine concentrations are unchanged in the extra-striatal brain regions, such as the frontal cortex, nucleus accumbens, nucleus amygdalae, hippocampus and hypothalamus (Morgan and Gibb, 1980; Ricaurte et al., 1980; Ohmori et al., 1993). The reasons underlying regional specificity are not known. Beside a decrease in dopamine concentration, decreased serotonin concentrations are also found in the rat (Table 4): This effect, however, is not restricted to the striatum (Ohmori et al., 1993). Interestingly, the neurotoxic effect of methamphetamine in the mouse appears to be specific for the nigrostriatal dopaminergic system (Table 3).

Beside the lowered concentrations of dopamine and its metabolites, diminished TH activity is also found (Table 3). These biochemical changes are accompanied by the degeneration of dopaminergic nerve endings (Ricaurte et al., 1982) and a transient diminished number of dopamine reuptake sites (Ikawa et al., 1994). Additionally, a smaller number of TH-immunoreactive neurones could be demonstrated in the SN (Kogan et al., 1976). Using the technique of microdialysis it has been demonstrated that multiple injections of methamphetamine [4 × 4mg/kg s.c. (subcutaneously) at 2 hourly intervals] lead to an initial massive liberation of dopamine in the striatum. This effect is negatively correlated with the histologically demonstrated loss of TH-immunoreactive neurons (O'Dell et al., 1991). Further, a gradual increase in extracellular glutamate has also been found (Abekawa et al., 1994).

The influence of the methamphetamine-induced neurochemical and histopathological changes described on the motor behaviour of the animals has not yet been systematically studied. The relevant literature does not mention any changes in spontaneous motor behaviour.

The neurotoxic effects of methamphetamine may be partially, and in some cases completely, prevented by inhibition of TH (Gibb and Kogan, 1979) and by dopamine receptor antagonists (Kogan et al., 1976; Gibb and Kogan, 1979; Nash and Yamamoto, 1992). The noncompetitive NMDA-receptor antagonist MK-801 and other, competitive, NMDA-receptor antagonists also appear capable of this inhibition (Sonsalla et al., 1989, 1991; Ohmori et al., 1993; Marshall et al., 1993). However, riluzole and lamotrigine, compounds that do not bind to any known glutamate receptor subtype, but inhibit the release of glutamate do not (Boireau et al., 1995). The neuroprotective effect of glutamate-receptor antagonists on the methamphetamine-model is probably related to the inhibition of glutamate-receptor regulated dopamine liberation. Using microdialysis, it was recently shown that dopamine-receptor antagonists such as SCH 23390 or eticlopride, and also the glutamate-receptor antagonist MK-801, could prevent the massive initial flood of dopamine in the striatum. However, MK-801 alone has no effect on dopamine liberation, while the dopamine-receptor antagonists lead to a release of dopamine by

**Table 3.** Neurotoxic effects of methamphetamine in the rat and the mouse

| Experimental animal | Experimental protocol | Neurotransmitter- and metabolite concentrations (% of normal values) | | | | | TH activity (% of normal values) | Reference |
|---|---|---|---|---|---|---|---|---|
| | | DA | DOPAC | HVA | 5-HT | 5-HIAA | | |
| Wistar-King-rats | 7.5 mg/kg[1] 4 × s.c. at 2 hr intervals. Killed 5 days after last injection. | 44 | 52 | 57 | 50 | 58 | – | Ohmori et al. (1993) |
| Transgenic mice | 25 mg/kg[1] s.c., single dose. Killed 1 week after last injection. | 16 | 24 | – | 100 | 100 | – | Cadet et al. (1994) |
| | 5 mg/kg[1] s.c., twice daily for 6 days; day 7 single dose of 15 mg/kg[1]; day 8 single dose 10 mg/kg[1]. Killed 1 week after last injection. | 43 | 56 | – | 100 | 100 | – | Sonsalla et al. (1991) |
| Swiss-Webster mice | 4 doses of 10 mg/kg[2] i.p. 17 Killed 3 days after last injection | 25 | 50 | – | – | – | 26 | Ricaurte et al. (1982) |

*DA* dopamine, *DOPAC* 3,4-dihydroxyphenylacetic acid, *5-HIAA* 5-hydroxyindoleacetic acid, *5-HT* serotonin, *HVA* homovanillic acid, *TH* tyrosine hydroxylase. *i.p.* intraperitoneal, *s.c.* subcutaneous, – not determined, [1] calculated as the hydrochloride, [2] calculated as the free base

antagonising pre-synaptic dopamine $D_2$-receptors and $D_2$-autoreceptors (Marshall et al., 1993).

### 2.2.2 The mechanism of neurotoxic action

The mechanism of the action of methamphetamine, which ultimately leads to the degeneration of the dopaminergic neurons, has not been finally established. Originally it was assumed that the dopamine which was released in a non-physiological fashion by large doses of methamphetamine was non-enzymatically converted to 6-OHDA (Seiden and Vosmer, 1984): A single large dose of methamphetamine (100mg/kg i.p.) apparently gave rise to a brief formation of 6-OHDA as indirectly demonstrated by HPLC determination (between 30 minutes and 2 hours following the injection of the methamphetamine), but this could not be confirmed using a direct mass-spectrometric method of detection (Karoum et al., 1993). Although 6-OHDA has not been measured in brain tissue this does not exclude the possibility that it could actually be synthetised in vivo. In fact, in vitro experiments have indeed demonstrated that dopamine efficiently converts to 6-OHDA in the presence of iron(II) and hydrogen peroxide via a Fenton-type reaction (Jellinger et al., 1995). Because of the rapid oxidation of 6-OHDA by iron(III), which is also produced by the Fenton reaction (Jellinger et al., 1995), it is difficult to detect 6-OHDA after administration of methamphetamine alone. Pretreatment with the MAO inhibitor pargyline (100mg/kg i.p.) and the COMT inhibitor pyrogallol (25mg/kg, i.p.) resulted in the HPLC detection of a 6-OHDA-like substance 30min after methamphetamine administration (Kita et al., 1995). Moreover, pargyline alone or in combination with pyrogallol exacerbated the long-lasting dopamine depletion induced by methamphetamine (50mg/kg, s.c.). These results indicate that simultaneous inhibition of MAO and COMT provides a cellular environment that encourages the autoxidation of dopamine to the 6-OHDA-like substance.

Since amphetamines on the one hand liberate dopamine in large amounts in a nonphysiological fashion, and on the other hand also inhibit its enzymatic breakdown by inhibition of MAO (Miller et al., 1980), "oxidative stress" – such as that which occurs following 6-OHDA – may be considered a possible causative factor in the neurotoxic action of methamphetamine. In fact, both of these pharmacological effects lead to increased autoxidation with increased hydrogen peroxide production, which can be converted to hydroxyl free radicals through catalysis by iron. This assumption is supported by various lines of indirect evidences. Prophylactic anti-oxidative treatment with vitamin C, vitamin E, alcohol and mannitol protects, in some cases completely, against the neurotoxic effects of methamphetamine (De Vito and Wagner, 1989). Treatment with inhibitors of MAO (Jarvis and Wagner, 1985) and SOD (De Vito and Wagner, 1989) as well as the additional administration of L-DOPA (Schmidt et al., 1985) accentuate its neurotoxic effects. It is also interesting that transgenic mice which carry the human Cu/Zn-SOD gene are protected against the neurotoxic effect of methamphetamine (Cadet et al., 1994).

The "excitotoxin-hypothesis" posits a possible alternative explanation to the excessive formation of hydroxyl free radicals (Sonsalla et al., 1989). This

hypothesis postulates that excessive liberation of excitatory amino acids (EAA) such as aspartic and glutamic acid leads to the death of nerve cells, and depends primarily on the observation that the neurotoxicity of methamphetamine can be prevented by glutamate-receptor antagonists. One hypothesis need not however necessarily exclude the other, as the latest findings indicate. The most direct evidence that excitotoxicity and "oxidative stress" may be sequential and interactive mechanisms leading to neuronal degeneration is the finding that NMDA exposure leads to superoxide radical generation in cultures of cerebellar neurons (Lafon-Cazal et al., 1993). In a more recent investigation it was shown that free radical spin traps, such as α-phenyl-N-tert-butylnitrone (PBN) and N-tert-butyl-α-(2-sulfophenyl)-nitrone (S-PBN), can attenuate excitotoxic lesions in vivo (Schulz et al., 1995a). These compounds react with unstable free radicals to produce more stable nitroxides. Pretreatment with S-PBN significantly attenuated striatal excitotoxic lesions in rat produced by NMDA, kainic acid, and AMPA. In a similar manner, striatal lesions produced by $MPP^+$, malonate, and 3-acetylpyridine were significantly attenuated by either S-PBN or PBN treatment. These results provide in vivo evidence for the involvement of free radicals in excitotoxicity.

Because hydroxyl radicals can alter the pre-synaptic potential of glutamate receptors it is likely that glutamate is liberated in a non-physiological fashion (Gilman et al., 1993), thus contributing to a vicious circle which leads to further cell damage. It is also interesting to note the recently described finding of the methamphetamine induced loss of ATP in the mouse striatum (Chan et al., 1994). Energy impairment could secondarily leads to slow excitotoxic neuronal death by increasing the sensitivity of EAA receptor activation (for review see Beal, 1992). It is possible, therefore, that perturbations of energy metabolism and excitotoxic effects might link the processes leading to the final dopaminergic toxicity.

### 2.3 The MPTP model

Another experimental model of PD is the so-called MPTP model. MPTP is now the most widely used and the best investigated model of PD (for reviews see Gerlach et al., 1991; Heikkila and Sonsalla, 1991; Tipton and Singer, 1993). During the period 1979 to 1982 observations were made on a number of young drug-dependent Californians who had injected a new "synthetic heroin" and had developed a serious and irreversible Parkinson syndrome (Davis et al., 1979). These patients exhibited all the symptoms typical of PD and responded well to treatment with L-DOPA and dopamine-receptor agonists. Analysis of the "synthetic heroin" showed it contained not only about 25% of the actual active agent 1-methyl-4-phenyl-4-propionoxypiperidine, but also up to 2.9% MPTP (Langston et al., 1983). The potential of this substance to produce the Parkinson syndrome was subsequently confirmed in various animal families (for reviews see Zigmond and Stricker, 1989; Gerlach et al., 1991; Heikkila and Sonsalla, 1991). Biochemical and histological investigations demonstrated that the MPTP-induced Parkinson syndrome in man exactly coincided with PD in all salient features (for a review see Gerlach et al., 1991). The

animal experimental MPTP syndrome is now considered to be the model which most accurately reproduces all aspects of PD in man.

### 2.3.1 Factors influencing the neurotoxic action of MPTP

*2.3.1.1 The kinds and families of laboratory animals used.* Neurological effects following systemic application of MPTP have been found in a variety of animal families, including monkeys, mice, dogs, cats, sheep and even goldfish (for a review see Zigmond and Stricker, 1989; Gerlach et al., 1991; Heikkila and Sonsalla, 1991; Tipton and Singer, 1993). Notable are the marked differences with regard to sensitivity to the neurotoxic action of MPTP. Table 4 gives an overview of MPTP dosages and their effects on striatal dopamine concentrations for the most commonly used laboratory animals. Even large doses of MPTP elicit only slight neurotoxic effects in rats and guineapigs. In order to produce dopamine losses similar to those seen in monkeys in even the most sensitive strain of mice, the C57/Black mouse, a 50-fold dose of MPTP is required.

The reasons for the differential sensitivities between animal families and subspecies are still not completely understood, however the differential pharmacokinetics of MPTP, and the differential distribution and excretion rate of its main metabolite $MPP^+$, may be chiefly responsible. Other factors influencing these species-differences such as neuromelanin, differences in the distribu-

**Table 4.** Relative toxicity of MPTP in various different families, species and strains of animals

| Animal family | Cumulative dose (mg/kg) | Dopamine concentration (% of normal values) | Reference |
|---|---|---|---|
| *Rodents* | | | |
| – Rat | | | |
| Sprague-Dawley | 151 | 77 (Caudate nucleus) | Przuntek et al. (1985) |
| – Guineapigs | 105 | 50 (Striatum) | Chiueh et al. (1984) |
| – Mouse | | | |
| C57/Black | 90 | 24 (Caudate nucleus) | Gerlach et al. (1993) |
| CF/1 | 80 | 60 (Striatum) | Riachi and Harik (1988) |
| Swiss-Webster | 410 | 35 (Striatum) | Weihmuller et al. (1989) |
| *Non-human primates* | | | |
| Common marmoset | 6.9–9.2 | 15 (Caudate nucleus) | Rose et al. (1990) |
| Rhesus monkey | 1.5 | 3 (Striatum) | Chiueh et al. (1984) |
| | 2.1–6.5 | 0.4 (Caudate nucleus)<br>0.5 (Putamen) | Pifl et al. (1988) |
| Squirrel monkey | 2 | 30 (Caudate nucleus)<br>15 (Putamen) | Irwin et al. (1990) |

The doses of MPTP are calculated as the free base

tion and localisation of MAO-subtypes in the brain, differences in dopamine metabolism and the anti-oxidant content of the nigro-striatal system, also appear to play a role (for reviews see Gerlach et al., 1991; Tipton and Singer, 1993).

*2.3.1.2 Intraindividual variability.* In addition to differential sensitivity between animal families and subspecies to the neurotoxic action of MPTP, there also appear considerable individual differences. These are particularly marked and striking in monkeys. Because of these individual differences in sensitivity, many laboratories do not have a standard scheme of dosage for producing a Parkinson-syndrome. Instead each animal is given a dose of MPTP adequate to initiate an assortment of Parkinsonian symptoms persisting over several weeks.

The causes of individual differences in sensitivity to MPTP among animals of the same species are not known, and have also received little systematic investigation. It is possible that individual variability in the sensitivity of the different dopaminergic neuronal systems and the biological variability in the function of compensatory mechanisms play a deciding role. Investigations in monkeys have indeed shown that in animals which show no symptoms the dopamine loss in the striatum (75%) is less than that in animals which do show symptoms (>95%) (Elsworth et al., 1989). Furthermore, in symptomatic animals extra-striatal dopamine losses suggest that the mesolimbic dopaminergic system is also lesioned (Elsworth et al., 1989; Pifl et al., 1990).

*2.3.1.3 The age of the animals.* Since PD is an illness occurring late in life, and because either exogenous or endogenous MPTP-like neurotoxins might possibly play a part in its pathogenesis, the interesting question arises whether older animals are more sensitive to the neurotoxic action of MPTP than younger ones. In fact, in mice such an age-dependency could indeed be observed (for a review see Heikkila and Sonsalla, 1989). Older animals are more severely damaged than younger ones, and additionally, showed less ability to achieve a functional recovery. Earlier results have been substantially confirmed in a systematic investigation of C57/Black mice with an age range from 2 to 24 months (Irwin et al., 1992). Between the second and 10th month of life (corresponding in man to youth and early adulthood) the increase in sensitivity to MPTP is greatest. Between the 10th and 16th months of life (corresponding in man to young adulthood to middle age) a further slight increase in sensitivity can be demonstrated, which however increases no further but rather decreases slightly till the 24th month of life. During the 22 month observation period there was no age-dependent decrease in dopamine concentrations such as that which occurs in man (Riederer and Wuketich, 1976). However, it is interesting that MAO-B activity shows a similar time-course as the age-dependent sensitivity to MPTP, but the sensitivity to $MPP^+$, in whose formation MAO-B is involved, is independent of the age of animals. A possible conclusion is the $MPP^+$ may not be the ultimate toxin (see 2.3.3). This conclusion is corroborated by an investigation in which the concentrations of MPTP and $MPP^+$ in various brain regions from NMRI and C57

Black mice and Sprague-Dawley rats were measured after systemic administration of MPTP (Nwanze et al., 1995): The tissue concentration of $MPP^+$ appeared not to be the determing factor for vulnerability of dopamine and noradrenaline neurons to MPTP, because equal concentrations of $MPP^+$ were found in regions showing marked as well as no neurotoxic effects of MPTP.

These age-dependent effects have been confirmed in primates. Young marmosets (common marmosets, callithrix jacchus; 6–8 months old) are more resistant than early adult (2–4 year-old) or late adult (8–10 year-old) animals (Rose et al., 1993): In order to obtain the same degree of severity of symptoms, young animals require higher doses of MPTP over a longer period. The effect of this in the young animals is a massive loss of dopamine from the striatum. In comparison to these young animals, the young adult and late adult animals cope better with functional disturbances.

*2.3.1.4 The influence of experimental design.* Beside the genus-dependent variations in sensitivity to MPTP, individual susceptibility and age, a number of other factors have a significant influence. Thus different methods of administration (the mode of injection – whether s.c. or i.p; number and frequency) of the same dose of MPTP may produce different results. This is demonstrated in Table 5 which displays examples of various experimental protocols.

The neurotoxic action of MPTP is potentiated by lesioning of the locus ceruleus. In the mouse this can be provoked by the additional systemic administration of the noradrenergic neurotoxin DSP-4 (Marien et al., 1993). In the squirrel monkey (saimiri sciureus) the same effect was shown after bilateral injections of 6-OHDA (Mavridis et al., 1991). Other pharmacological potentiating interventions include additional administration of diethyldithiocarbamate (DDC) (Corsini et al., 1985), alcohol, and acetaldehyde (Corsini et al., 1987). However, these approaches have been restricted to mice. All these substances affect neurotoxin-metabolising enzymes, and this is the mechanism by which they accentuate the neurotoxic effect of MPTP. Thus, DDC is a substance which chelates copper, and by so doing inactivates a range of copper-containing enzymes such as SOD and aldehyde dehydrogenase; high doses of acetaldehyde inhibit aldehyde dehydrogenase.

### 2.3.2 Neurotoxic action

One of the central problems concerning the MPTP model is the question of the longevity of MPTP effects. Although initially no long-term investigations were available, it was concluded on the basis of the human Parkinsonian syndrome that the neurotoxin also led to a permanent damage of the nigrostriatal system and a corresponding functional disturbance in animals. Long-term studies involve an enormous experimental investment, and in addition, the MPTP-damaged monkeys, because of their massively compromised motor behaviour, require intensive care. Thus a few systematic long-term studies have been undertaken. What is striking about their results is the discrepancy between the neurochemical and behavioural effects on rodents and on monkeys.

**Table 5.** Experimental factors influencing the neurotoxic effect of MPTP on the C57/Black mouse

| Cumulative dose (mg/kg as free base) | Experimental protocol | Dopamine loss (% of normal values) | References |
|---|---|---|---|
| *Mode and frequency of the injections* | | | |
| 300 | 30 mg/kg i.p. once a day for 10 days. Animals killed 12 days after the last treatment. | 80 | Heikkila et al. (1989) |
| 80 | 20 mg/kg i.p. 4 times at 2-hour intervals. Animals killed 12 days after the last treatment. | 80 | Heikkila et al. (1989) |
| 40 | 10 mg/kg i.p. 4 times at 2-hour intervals. Animals killed one week after the last treatment. | 40 | Marien et al. (1993) |
| 40 | Single subcutaneous injection. Animals killed 12 days after the last treatment. | 80 | Heikkila et al. (1989) |
| 80 | 40 m/kg subcutaneously 16 hours apart. Animals killed 4 weeks after the last treatment. | 79 | Sundström et al. (1990) |
| *Time after which the effect is measured* | | | |
| 300 | 30 mg/i.p. daily for 10 days. | | Ricaurte et al. (1986) |
| | 1 week after the last treatment | 66 | |
| | 3 weeks after the last treatment | 70 | |
| | 2 months after the last treatment | 35 | |
| | 4 months after the last treatment | 14 | |

All experimental animals weighed at least 25 g, and were aged between 6 and 10 weeks; *i.p.* intraperitoneal

Mice, in spite of the massive damage to the dopaminergic system caused by the application of MPTP together with acetaldehyde, already show normal spontaneous behaviour 24 hours later, and at seven days post-treatment the animals are no longer hypokinetic despite a 93% loss of dopamine (Zuddas et al., 1992). This dopamine deficiency is maintained four months following MPTP treatment. Similar results were described with marmosets – a species of monkey at a lower evolutionary level of development – (Ueki et al., 1989). Although dopamine was still markedly depleted in the striatum 12–18 months after the application of MPTP (95% in the caudate nucleus; 93% in the putamen), the motor performance of the animals had recovered, and the animals exhibited normal motor behaviour. Two possible mechanisms responsible for this were discussed: first, that there might be processes for regeneration of damaged nerve-endings or collatered formation of intact neurons (see for example Gaspar et al., 1993); or second, the occurrence of compensatory mechanisms (plasticity of the brain).

In higher primates such as the baboon (papio papio) or rhesus monkeys (macaca mulatta) a permanent Parkinsonian syndrome is observed. Baboons injected weekly with 0.4–0.5 mg/kg MPTP intravenously up to the age of 20 months, exhibit a stable Parkinsonian syndrome, which is observed even 16 months after the last dose of MPTP (Hantraye et al., 1993). Old rhesus monkeys (over 23 years of age) which had been injected with MPTP (0.4 mg/kg) bilaterally into the cerebral artery similarly developed a stable Parkinsonian syndrome which persisted up to a maximum of 45 days after the lesion. A 95% loss of total motor activity could still be demonstrated 12 months after the lesion (Smith et al., 1993). In neither experiments no neurochemical analyse were performed, thus it is not possible to comment on the neurochemical correlation of this functional disturbance.

The MPTP-induced Parkinsonism can be treated both in man and in non-human primates with L-DOPA and dopamine-$D_2$-receptor agonists (e.g. Davis et al., 1979; Burns et al., 1983; Bédard and Boucher, 1989; Close et al., 1990). The non-competitive NMDA-receptor antagonist MK-801 applied systemically had no effect on improving the symptoms in monkeys (Crossman et al., 1989; Close et al., 1990). On the other hand, the competitive NMDA-receptor antagonists CPP (Löschmann et al., 1991) and CGP 40.116 (Wüllner et al., 1992) potentiated the action of L-DOPA. The effects of the AMPA-receptor antagonist NBQX is controversial and has not been definitely established (Klockgether et al., 1991; Luquin et al., 1993). Controversy also surrounds the potentiation of dopamine $D_1$ and $D_2$ agonist effects by the systemic application of glutamate antagonists (see for a review Starr, 1995).

Prophylactic treatment with MAO-B inhibitors (for example selegiline) and dopamine-uptake inhibitors (for example nomifensine) protects monkeys from the neurotoxic action of MPTP by preventing it from being metabolised to $MPP^+$ or from being taken up into the dopaminergic neurons (for a review see Gerlach et al., 1991). The neuroprotective action of the antioxidants vitamin C and vitamin E has also not been established (for a review see Gerlach et al., 1991). In marmosets which were treated with high doses of vitamin C (100 mg daily) and vitamin E (2,350 mg daily) no protective effect

against the MPTP-induced depletion of dopamine in the striatum could be demonstrated (Mihatsch et al., 1991). The neurotoxic effects of MPTP could, however, be partially prevented by prior administration of SH-containing antioxidants such as cysteamine or dimercaprol (Oishi et al., 1991). A combination of coenzyme Q(10) and nicotineamide protected against both mild and moderate depletion of dopamine (Schulz et al., 1995a). In the MPTP regimen which produced mild dopamine depletion nicotineamide or the free radical spin trap S-PBN were also effective. These treatment, however, afforded no protection against a MPTP regimen which produced severe dopamine depletion. These results might explain the reported differences in regard to the neuroprotective effects of antioxidants. Partial protection has also been demonstrated by ergot alkaloids (Bernocchi et al., 1993), lipid membrane components such as GM1-gangliosides (Fazzini et al., 1990), inhibitors of neuronal nitric oxide synthase (Schulz et al., 1995b), intracerebral chronic administration of neurotophins such as BDNF (brain-derived neurotrophic factor) and FGF (fibroblast growth factor) (Otto and Unsicker, 1990; Tsukahara et al., 1995), and calcium channel blockers such as nimodipine (Gerlach et al., 1993; Kupsch et al., 1995). The neuroprotective action of NMDA antagonists, which also interfere with intracellular calcium influxes, is not yet established: While MK-801 showed no neuroprotective action in mice (Kupsch et al., 1992; Sonsalla et al., 1992), the NMDA antagonist CPP protects SN neurons from MPTP-induced degeneration in primates (Lange et al., 1993).

*2.3.2.1 Behavioural changes.* Monkeys treated with MPTP develop motor disturbances comparable to those in man (for reviews see Stern, 1990; Gerlach et al., 1991). The most prominent of these are akinesia and rigidity; resting tremor on the other hand is only seen in isolated instances. The precise appearance of symptoms varies, depending on the species, age and dosage. MPTP-syndromes have been described, among others, in rhesus monkeys (Burns et al., 1983; Smith et al., 1993), in squirrel monkeys (Irwin et al., 1990), in macaques (Crossman et al., 1989), in baboons (Hantraye et al., 1993) and above all in marmosets (Ueki et al., 1989; Russ et al., 1991). The quantitation of the symptoms in monkeys is achieved using modified PD scales. Various types of apparatus have also been applied to measure spontaneous locomotor activity, for example cages with a light-barrier, with which one can gain a measure of the mean total activity per unit time.

MPTP-damaged mice, after the initial acute toxity effects such as mydriasis, piloerection, hypersalivation and clonic seizures have worn off (15–30 minutes) recover normal spontaneous behaviour relatively rapidly (e.g. Sundström et al., 1990; Zuddas et al., 1992). Hypokinesia is scarcely noticeable (Weihmuller et al., 1989; Zuddas et al., 1992), although decreased locomotor activity (−66%) has been described (Sundström et al., 1990). Remarkable, however, is the motor behaviour of MPTP-damaged mice which are injected with small doses of haloperidol (0.2 mg/kg i.p.) (Weihmuller et al., 1989). At this dosage, haloperidol elicits no changes in motor behaviour in the undamaged animal, but in the MPTP-lesioned animal one can clearly measure a distinct deterioration of somatosensory orientation. This normalises itself –

in parallel with the striatal dopamine content – over a period of three to five months. Additionally, the MPTP-damaged animal exhibits akinesia and catalepsy; and these motor disturbances are indeed still present after five months (Weihmuller et al., 1989). These changes in motor behaviour are attributed to the supersensitivity of dopaminergic neurons produced by MPTP.

*2.3.2.2 Histopathological changes.* The literature contains only one report of a post mortem study on a heroin-addict who suffered from an MPTP-induced Parkinsonian syndrome. The histological investigation showed a selective destruction of dopaminergic neurones of the SN pars compacta (Davis et al., 1979). In the majority of cases while there a selective destruction of SN neurons has been described in MPTP-treated non-human primates as well (e.g. Burns et al., 1983; Ueki et al., 1989; Mavridis et al., 1991; Bernocchi et al., 1993; Hantraye et al., 1993); more detailed examination show that other regions of the brain are also affected (Mitchell et al., 1985; German et al., 1988; Gibb et al., 1989; Forno et al., 1993). Especially in older animals, a loss of neurons is also found in the locus ceruleus (Mitchell et al., 1985; Forno et al., 1993). Immunocytochemical methods also demonstrated that even in younger animals losses of TH-immunoreactive neurons has occurred in the VTA and the hypothalamus (German et al., 1988): quantitative evaluation showed that a cumulative dose of 1.75–4.59 mg/kg MPTP in macaques led to a cell loss between 46% and 93% in the SN pars compacta, though only 28% to 57% in the VTA. This procedure leads to a stable Parkinsonian syndrome with a more than 99% loss of dopamine in the striatum.

The presence of Lewy bodies – an equally characteristic pathological marker of PD in man – has not been definitely established in MPTP-damaged non-human primates. In old monkeys eosinophil inclusion-bodies have been diagnosed to occur in those brain structures in which Lewy-bodies are found in humans (Forno et al., 1993), but the significance of this finding is difficult to judge because of the differences in their morphological and immunocytochemical characteristics.

Systematic investigations, including all brain regions, are not available for the mouse. Immunocytochemical analyses using antibodies against TH show an average loss of 40% in the SN of MPTP treated C57/Black mice (cumulative dose 80 mg/kg) (e.g. Date et al., 1990; Kupsch et al., 1992), and there was additionally a 17% loss in the VTA (Date et al., 1990). This degree of damage leads to a substantial depletion (−85%) of dopamine in the striatum of the affected animals (Date et al., 1990). Semi-quantitative investigations, in which as well as the immunocytochemical methods Nissl-staining was used, confirmed these results (Seniuk et al., 1990). Further, it was shown that neurons in the locus ceruleus and the hypothalamus were also damaged (Seniuk et al., 1990).

*2.3.2.3 Neurochemical changes.* MPTP produces a series of neurochemical changes in primates and rodents. Table 6 summarises the salient features of the results obtained in monkeys. Decreased concentrations of dopamine and

**Table 6.** Neurochemical effects of MPTP in monkeys

| Neurochemical parameter | Brain region (% of normal values) | | | | |
|---|---|---|---|---|---|
| | Caudate nucleus | Putamen | Globus pallidus | Nucleus accumbens | Ventral tegmental area |
| Dopamine | 18.9[a] | 4.0[a] | 50.0[b] | 28.3[b] | 40.8[b] |
| 3,4-Dihydroxyphenylacetic acid | 25.9[a] | 8.7[a] | 46.0[b] | 22.8[b] | 23.3[b] |
| Homovanillic acid | 20.4[a] | 7.1[a] | 14.3[b] | 25.5[b] | 38.3[b] |
| Noradrenaline | 100.0[a] | 42.9[a] | 150.0[c] (n.s.) | 103.0[c] (n.s.) | 128.0[c] (n.s.) |
| Serotonin | 36.4[a] | 19.3[a] | 148.0[c] (n.s.) | 165.0[c] | 94.0[c] (n.s.) |
| 5-Hydroxyindoleacetic acid | 18.8[a] | 12.7[a] | – | – | – |
| Tyrosine hydroxylase activity | – | – | 24.6[b] | 25.8[b] | 43.4[b] |
| [$^3$H]Mazindol-binding density | 15.7[d] | 14.6[d] | – | 65.4[d] | – |

– not determined, *n.s.* not significantly different from normal values: all other values are significant, [a] Russ et al. (1991), [b] Pifl et al. (1990), [c] Pifl et al. (1991), [d] Ueki et al. (1989)

its metabolites, diminished TH activities and fewer dopamine-uptake sites in the striatum and the globus pallidus show what damage has been done to the nigro-striatal dopaminergic system. By analogy with the histological findings, these changes are not restricted to that system (Table 6). Reduced noradrenaline concentrations are found in cortical and limbic areas (for example −79% in the motor cortex, −78% in the supplementary cortex, −63–75% in the frontal cortex, −59% in the entorhinal cortex, −69% in the horn of Ammon) (Pifl et al., 1991). In contrast to the neurotransmitters dopamine, noradrenaline and serotonin, neuropeptides seem to be less affected by MPTP. The concentrations of methionine- and leucine-enkephalins as well as cholecystokinin, substance P and neurotensin are unchanged at least in the basal ganglia of MPTP-treated marmosets (Taylor et al., 1991).

The question of whether MPTP provokes an interregional dopamine depletion in the striatum as one sees it in the brains of patients with PD has given contradictory answers. In the marmoset (Russ et al., 1991) and the squirrel monkey (Moratalla et al., 1992) the putamen is more severely affected than the caudate nucleus, but in the macaque (Alexander et al., 1991) the results were the other way round. These differences do not appear to be species-dependent, however, because in the marmoset different experimental protocols have led to divergent results (Russ et al., 1991; Taylor et al., 1991).

By analogy with some of the results in PD, with the MPTP model in macaques one finds a diminished [$^3$H]spiperone-binding density of the $D_2$-dopamine receptor (−40% in the putamen, −25% in the caudate nucleus), but not a diminished [$^3$H]SCH-23390-binding density of the $D_1$-receptor (Alexander et al., 1991). This is limited to the lateral portions and can be explained on the basis of the supersensitivity of the post-synaptic dopamine $D_2$-receptor. Treatment with the dopamine $D_2$-receptor agonist (+)-PHNO results in a 40–70% decrease in the [$^3$H]spiperone-binding sites, which above all is based on the down-regulation of the dopamine $D_2$-receptor by the agonist.

The essential features of the neurochemical effects observed in primates under MPTP-treatment, such as the depletion of dopamine in the nigro-striatal and mesolimbic systems or of noradrenaline in cortical regions can also be demonstrated in various strains of mice (e.g. Heikkila et al., 1989; Weihmuller et al., 1989; Date et al., 1990; Seniuk et al., 1990; Sundström et al., 1990; Gerlach et al., 1993).

### 2.3.3 The mechanism of the neurotoxic action

The precise pathological mechanism of the neurodegeneration caused by MPTP has not been definitely established. Cells, including neurons, die by necrosis or programmed cell death (e.g. Vaux, 1993), which can be differentiated by distinct morphological and biochemical features (e.g. Buja et al., 1993). Programmed cell death having the morphology of apoptosis plays a critical role in development and morphogenesis. In vitro data showing that $MPP^+$ can cause apoptotic cell death in cerebellar granule cells (Dipasquale et al., 1991), in PC12 cell lines (Hartley et al., 1994), and in fetal mesencephalic

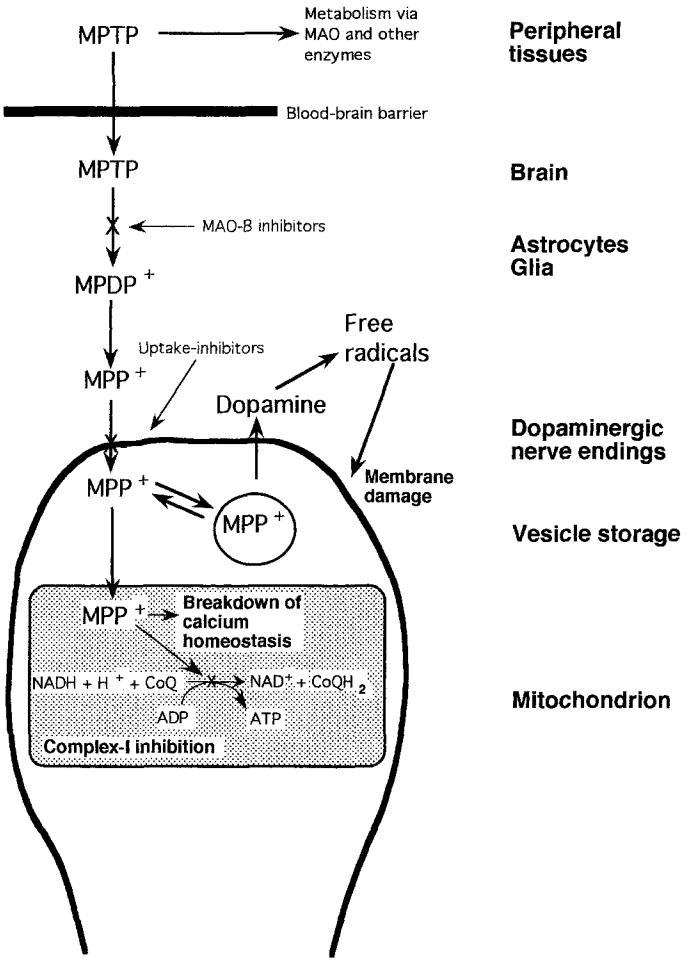



**Fig. 1.** Pathobiochemical mechanisms of MPTP-induced neurotoxic action on dopaminergic neurons (adapted from Gerlach et al., 1996a). Further details are discussed in the text. *CoQ* ubiquinone, $CoQH_2$ ubiquinol, *MAO* monoamine oxidase, $MPDP^+$ 1-methyl-4-phenyl-2,3-dihydropyridinium ion, $MPP^+$ 1-methyl-4-phenylpyridinium ion, *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

neurons (Mochizuki et al., 1994) have been reported. However, recent investigations in mice provide no evidence that MPTP kills neurons by apoptosis in vivo at one and 2 days after MPTP administration (Jackson-Lewis et al., 1995).

What is certain is that the mechanism of MPTP neurotoxicity involves several steps (see Gerlach et al., 1991; Tipton and Singer, 1993 for recent reviews; Fig. 1), two of them being potentially important to explain the preferential vulnerability of the nigral dopaminergic neurons projecting to striatal regions (caudate nucleus, putamen). In a first step MPTP, which readily penetrates the brain, is converted, possibly in the glia by MAO-B to $MPP^+$, the probably ultimate toxic agent. The second step is a fairly selective uptake of $MPP^+$ by dopaminergic terminals in the striatum via the monoamine trans-

porter and a subsequent intraterminal vesicular storage. What has not yet been established, however, are the processes by which $MPP^+$ or other toxic metabolites damage the nerve cells so that they become non-functional and eventually die. The following theories have crystallised from the immense amount of experimental data (for a review see Gerlach et al., 1993; Tipton and Singer, 1993):

– *$MPP^+$ damages dopaminergic neurons through inhibition of mitochondrial respiratory chain enzymes and subsequent ATP-exhaustion*: the selective inhibition of complex-I activity by high concentrations of $MPP^+$ (1–2 mM), which has been shown in vitro in heart and liver mitochondria, might on its own be considered sufficient cause of the nerve degeneration. However, the recent in vivo findings that acute MPTP treatment has no effect on any of the enzymes of the respiratory chain (Gerlach et al., 1996a) suggest that factors other than mitochondrial impairment may be involved in MPTP neurotoxicity.

– *Dopaminergic neurons are more severely exposed to "oxidative stress"*. Obata and Chiueh (1992) have used the technique of microdialysis to demonstrate in vivo that $MPDP^+$ (1-methyl-4-phenyl-2,3-dihydropyridinium ion, a further MPTP metabolite) as well as $MPP^+$ on intrastriatal application are briefly able to potentiate dopamine liberation from dopaminergic neurons in the rat, and indirectly that there was a brief production of hydroxyl free radicals, as evidenced by the reaction products formed with salicylic acid. In in vitro investigations using ESR (electron spin resonance) spectroscopy it could be shown that superoxide free radicals are formed during the metabolism of MPTP (e.g. Rossetti et al., 1988; Zang and Misra, 1992). Additionally, Adams et al. (1993) demonstrated that hydrogen peroxide and hydroxyl radicals are also products of the interaction of $MPP^+$ with complex I. We may assess the finding that MPTP leads to a liberation of iron(II) and iron(III) in the SN of monkeys (Temlett et al., 1994) as further evidence for the involvement of hydroxyl free radicals in the MPTP-induced neurodegeneration. Increased free iron concentrations were demonstrated not only in the damaged dopaminergic neurons but also in the surrounding matrix and glial cells. The animal experimental MPTP model provided further indirect evidence for the presence of "oxidative stress" in rodents, such as increased lipid peroxidation (Rios and Tapia, 1987) and diminished concentrations of the anti-oxidants vitamin C, GSH and uric acid (Riederer et al., 1988; Oishi et al., 1991; Desole et al., 1993). However, these findings cannot be clearly shown in non-human primates. For example, the most recent investigations in the common marmoset did not show any significant alterations of the endogenous antioxidants GSH and ubiquinol (the reduced form of coenzyme Q) one week after cessation of MPTP administration (Gerlach et al., 1996a). Although transient effects can not be ruled out, we believe that at least a primary oxidative damage to complex I by $MPP^+$ is excluded, because this would lead to an irreversible damage to complex I, but no inhibition of complex-I activity was found in our experiments ex vivo one week after the last toxine exposure (Gerlach et al., 1996a). In addition, our data cannot

provide support for the proposed hypothesis that cellular damage to nigrostriatal neurons is caused primarily by inhibition of ATP synthesis due to specific binding of $MPP^+$ to the rotenone-sensitive site of mitochondrial complex I (Bates et al., 1994). Following this assumption, a considerable decrease in the levels of GSH would be expected, as ATP is required for the synthesis of GSH (e.g. Mithöfer et al., 1992). In conclusion, these results suggest that other factors than mitochondrial impairment and/or "oxidative stress" may be involved in MPTP neurotoxicity in primates. Alternatively, the results imply that the $MPP^+$ is not the ultimate neurotoxin leading to dopaminergic cell death following systemic MPTP treatment.

– *The neurotoxic effect of MPTP is caused by a disturbance of calcium homeostasis.* The calcium hypothesis postulates that whenever pathologically increased intracellular calcium concentration occur, there will be an uncontrolled stimulation of calcium-dependent enzyme reactions, and that these will lead to altered cell function and the destruction of cellular structures (Siesjö, 1990). For example, the activation of calpains I & II lead to changes in the cyto-skeleton; activation of protein kinase C and nitric oxide synthase leads to the formation of toxic free radicals; activation of phospholipase $A_2$ leads to the degradation of phospholipid membranes. The fatty acids liberated in this process, for example arachidonic acid, move into the extracellular space where further breakdown processes convert them into free radicals. A vicious circle is thus created whereby the cell-damaging mechanisms are created and even potentiated. A collapse of calcium homeostasis within the mitochondria of dopaminergic nerve cells can be provoked not only by damage of the mitochondrial membrane by a free-radical mechanism but also by the inhibition of the mitochondrial respiratory chain enzymes. It was originally assumed that excessive calcium influx into neurons was caused by "voltage-dependent" ion channels, but a calcium influx coupled to the NMDA receptor and/or free radical-induced membrane damage could equally lead to a breakdown of calcium homeostasis. Thus it can be argued that the breakdown of intraneuronal calcium homeostasis represents an ultimate pathobiochemical mechanism which may also occur as the result of these previously described mechanisms. In the light of this hypothesis it appears probable that the effects of various neurotoxins which exert their actions via a variety of different pathological mechanisms could all be prevented by calcium-channel blockers. In fact, in animal experiments using the MPTP model in the mouse and in the monkey, a neuroprotective effect was indeed demonstrated with the calcium-channel blocker nimodipine (Kupsch et al., 1995, 1996). Similarly, glutamate antagonists such as NMDA receptor blockers partially prevent MPTP-induced neurotoxicity in non-human primates (Zuddas et al., 1992; Lange et al., 1993).

### 2.4 Intracerebral administration of $MPP^+$

Because rats are fairly resistant to the neurotoxic effects of MPTP, and the toxic metabolite $MPP^+$ cannot cross the blood-brain-barrier, the action of intracerebrally injected $MPP^+$ has also been investigated.

#### 2.4.1 Neurotoxic effects

Injection into the SN (Heikkila et al., 1985) and the median forebrain bundle (Altar et al., 1986) as well as intrastriatal perfusion (Obata and Chiueh, 1992) all lead to a massive loss of dopamine in the striatum. Unilateral injections of $MPP^+$ (10, 17.5 or 25 μg) into the medial forebrain bundle however also induce an 83–98% loss of dopamine from the nucleus accumbens on the same side which is not distinguishable from that demonstrated in the striatum (Altar et al., 1986). $MPP^+$ at the highest dose additionally induces a 60% loss of GABA from the SN on the same side (Altar et al., 1986). Apparently, $MPP^+$ destroys not only the nigro-striatal and mesolimbic dopaminergic neurons but also GABA-ergic striatal neurons. This is also expressed in the atypical circling behaviour of the lesioned animals (Altar et al., 1986): neither apomorphine (0.25 mg/kg i.p.) nor L-DOPA (10 mg/kg i.p.) elicit circling behaviour, but D-amphetamine (1.5 mg/kg i.p.) induces robust ipsilateral rotational motion.

The non-specific toxicity of $MPP^+$ is also confirmed by histological studies. $MPP^+$ (estimated amount 0.5–10.8 nMol) applied to the SN by iontophoresis leads to a general destruction of neurons and glial cells with necrotic defects and vacuoles in various different neurons, membrane damage and gliosis (Ter Horst et al., 1992). These effects are however difficult to reconcile with the findings of a selective uptake of $MPP^+$ into dopaminergic neurons by the dopamine-carrier system which thus explains the selective action of MPTP on dopaminergic neurons.

#### 2.4.2 The mechanism of the neurotoxic actions

As previously discussed, $MPP^+$ could damage neurons through inhibition of mitochondrial respiratory chain enzymes and subsequent ATP-exhaustion. Further, the generation of hydroxyl free radicals could lead to the non-specific destruction of neuron- and glial membranes, thus explaining the observed histological findings.

Acute administration of 10 mM $MPP^+$, either through a microdialysis probe (Obata and Chiueh, 1992) or by direct intrastriatal injection (e.g. Ballarin et al., 1989), causes a dramatic release of dopamine (30-fold compared to the baseline level) and a decrease of its metabolites DOPAC and HVA, lasting for more than two hours (Obata and Chiueh, 1992). A similar effect could be elicited by rotenone (Santiago et al., 1995), a typically inhibitor of complex I, and $MPDP^+$ (Obata and Chiueh, 1992), suggesting that the dopamine uptake system may have low selectivity. The $MPP^+$-induced dopamine release is voltage-sensitive and calcium-dependent (see Obata and Chiueh, 1992). Moreover, this dopamine releasing action of $MPP^+$ and $MPDP^+$ is dose-dependent (1 to 10 mM); in contrast, MPTP failed to increase dopamine levels in the dialysate of the striatum (Obata and Chiueh, 1992). Two days after the lesion with $MPP^+$, however, a 40% decrease in extracellular dopamine levels were measured, which lasts for at least 60 days (Espino et al., 1995). A similar effect could be induced with 6-OHDA (Espino et al., 1995). However, the mechanisms underlying the neurotoxic actions of $MPP^+$ and 6-OHDA must be different, as has been suggested by the study of the

time-course, the recovery from the lesions and the affectation of the SN neurons (Espino et al., 1995).

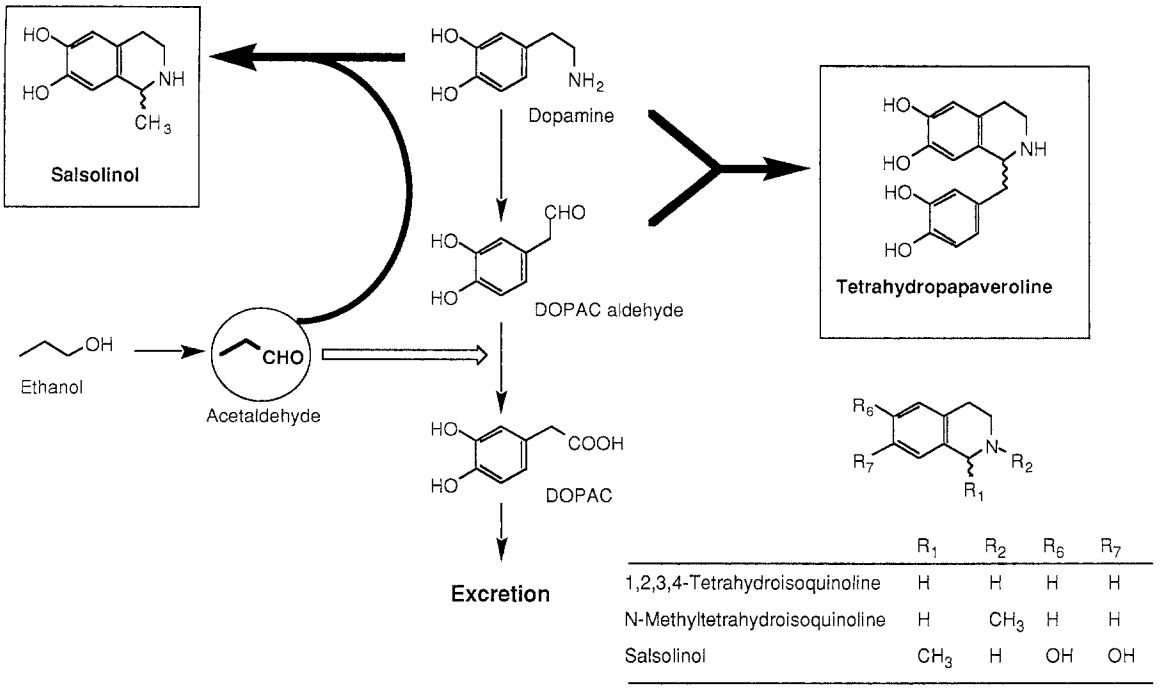
In addition to the liberation of dopamine, $MPP^+$ at 10mM maximally stimulated glutamate and aspartate release to 230- and 68-fold of baseline, respectively (Carboni et al., 1990). This release could not be observed with 1mM $MPP^+$. Pretreatment with the NMDA receptor antagonists MK-801 (5mg/kg i.p.) prevented the $MPP^+$-induced release of the EAA aspartate and glutamate. In contrast, MK-801 had no effect on dopamine release either induced by 1 or 10mM $MPP^+$ (Carboni et al., 1990). These results suggest that $MPP^+$-induced dopamine and EAA release are independently regulated processes. Because NMDA receptors are able to prevent $MPP^+$ toxicity in the SN (Turski et al., 1991), it has also been hypothesized that $MPP^+$ produced neuronal impairment of energy metabolism, which may result in membrane metabolism and excitotoxic neuronal degeneration (Storey et al., 1992).

### 2.5 Administration of MPTP-like synthetic compounds

The ability of MPTP to cause a parkinsonian condition has led to suggestions that there may be a naturally occuring or environmental toxin that causes PD. The extent to which such a compound might resemble MPTP in its actions is generally not specificed, although it is often tacitly assumed that activation by MAO-B and inhibition of mitochondrial function by the activated metabolite would be an important feature. Several compounds have been suggested as potential candidates for endogenous neurotoxins including 4-phenylpyridine (Snyder and D'Amato, 1985), paraquat (Lambert and Bondy, 1989), 1,2,3,4-tetrahydroisoquinoline and its derivatives (Niwa et al., 1987), and β-carbolines (Collins and Neafsey, 1985). However, although 4-phenylpyridine, which is present in several commonly used spices and also occurs in some industrial emission, is a substrate for MAO-B (Sullivan and Tipton, 1992), it has not been found to be acutely neurotoxic (e.g. Irwin et al., 1987). Further, the toxic actions of paraquat are restricted to the periphery because, unlike MPTP, it does not readily penetrate the brain (Lambert and Bondy, 1989).

#### 2.5.1 Tetrahydroisoquinolines

It has been suggested that 1,2,3,4-tetrahydroisoquinoline alkaloids (Fig. 2) may be endogenous toxins, leading to PD by a mechanism similar to that of MPTP. Several of these compounds have been found in mammalian brain and also in several foods and beverages (for a review see Dostert et al., 1988). Representative derivatives are 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) formed from dopamine and actealdehyde and tetrahydropapaveroline formed from dopamine and its metabolite DOPAC aldehyde (Fig. 2). Salsolinol possesses an asymmetric center at C-1 and exists as R and S enantiomers. R-Salsolinol is enantio-specially synthesized in the human brain by condensation of dopamine with pyruvic acid to 1-carboxylsalsolinol, followed by decarboxylation and reduction (Dostert et al., 1988). Using the microdialysis technique it was shown that the MPTP-like N-methyl-(R)-salsolinol is formed in the rat brain by N-methylation of the R-




| | $R_1$ | $R_2$ | $R_6$ | $R_7$ |
|---|---|---|---|---|
| 1,2,3,4-Tetrahydroisoquinoline | H | H | H | H |
| N-Methyltetrahydroisoquinoline | H | $CH_3$ | H | H |
| Salsolinol | $CH_3$ | H | OH | OH |

**Fig. 2.** Biosynthesis of tetrahydroisoquinolines. *DOPAC* 3,4-dihydroxyphenylacetic acid

enantiomer of salsolinol (Maruyama et al., 1992). Both of these enantiomers were also found predominantly in the human brain (Deng et al., 1995).

*2.5.1.1 Neurotoxic effects of N-methyl-salsolinol.* Unilateral stereotactic injection of N-methyl-(R)-salsolinol into the rat striatum leads to a behavioural, neurochemical and pathological condition similar to PD. The animals exhibit hypokinesia, a stiff tail, a limb twitching at rest and postural disturbances associated with a decrease in the concentration of dopamine and the activity of its synthesizing enzyme TH in the nigro-striatal system and a reduction in TH-immunoreactive neurons in the SN (Naoi et al., 1996).

*2.5.1.2 The mechanism of the neurotoxic action.* Analogous to the mechanisms underlying the neurotoxic effects of MPTP, the crucial step in the activation of N-methyl-(R)-salsolinol should be the oxidation by MAO. Although it has been recently reported both a non-enzymatic (Maruyama et al., 1995b) and an enzymatic oxidation of this compound, the enzymatic reaction could not be blocked by MAO inhibitors but was sensitive to semicarbazide (Naoi et al., 1995b). These results indicate that the semicarbazide-sensitive aminie oxidase is involved in the bioactivation of N-methyl-(R)-salsolinol. The resulting product 1,2-dimethyl-6,7-dihydroisoquinolinium ion, which is structurally related to $MPP^+$, has been shown to accumulate in the SN and striatum of rats injected with N-methyl-(R)-salsolinol (Naoi et al., 1996). Using human dopaminergic neuroblastoma SH-SY5Y cells it was demonstrated that this metabolite is selectively transported via the dopamine uptake system

(Takahashi et al., 1994). In rats, the intrastriatal perfusion leads to the liberation of dopamine as well as a reduction in its catabolism (Maruyama et al., 1995a). In vivo and in vitro investigations point to an involvement of hydroxyl radicals in the N-methyl-(R)-salsolinol-induced neurotoxicity (Maruyama et al., 1995a). Studies on isolated PC12 cells have shown that the bioactivated 1,2-dimethyl-6,7-dihydroisoquinolinium ion is a potent toxin, depleting ATP and being more potent than its precursor N-methyl-(R)-salsolinol (Naoi et al., 1995a).

### 2.5.2 β-Carbolines

Due to its structural similarity to MPTP the β-carbolines (synonyme norharmanes) have also been suggested as possible endogenous toxins leading to parkinsonism (Collins and Neafsey, 1985). They may be formed in vivo from condensation between tryptophan derivatives and aldehydes. Specifically, the 1,2,3,4-tetrahydro-β-carboline (THBC) has been detected by mass spectroscopy in the rat brain and adrenal tissue (Barker et al., 1981). It has been recently shown in vivo that also the aldehyde chloral (which is therapeutically given as an hypnotic) as well as a metabolite of trichloroethylene ("tri", a solvent widely used in industry) rapidly react with endogenous tryptamine to form the new 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline (TaClo, *tryptamine* and *chloral*) (Bringmann et al., 1995; Fig. 3). Due to its high lipophilicity TaClo readily penetrates the brain (Bringmann et al., 1995), as has been shown in rats after systemic administration (4 mg/kg i.p. for six days). However, because of its rapid metabolism only low concentrations of TaClo have been detected in the rat brain.

*2.5.2.1 Neurotoxic effects.* Owl monkeys (Aotus trivirgatus) chronically treated with high daily doses of 1-methyl-THBC (5–50 mg, either i.v. or i.p.) develop an acute motor behaviour comparable to that of MPTP-treated animals, but did not cause persistent motor disturbance nor a loss of nigral cells and striatal dopamine (Collins and Neafsey, 1985). However, 1-methyl-THBC exposure resembled MPTP in reducing DOPAC levels.

Intranigral injections of the N-methylated β-carboline 2-methyl-norharman (closely structurally resemble $MPP^+$) into the SN or median forebrain bundle of rats results in a depletion of striatal dopamine and its metabolites. Three weeks after intranigral injection of the β-carboline (137 μg 2-methyl-norharman iodide) striatal dopamine, DOPAC and HVA concentrations ipsilateral to the injection site are reduced 41–64% compared to vehicle-injected controls (Neafsey et al., 1989). However, the lesion produced by 2-methyl-norharman appeared to be non-specific, affecting dopaminergic and non-dopaminergic cells and fibers. Histologically, large lesions and gliosis were apparent under light microscopic examination (Neafsey et al., 1989).

In contrast to 1-methyl-THBC TaClo showed neurotoxic effects in motor behaviour of rats (Sontag et al., 1995): The subchronic i.p. injection of a daily dose of 0.2 mg/kg over a seven week period leads to an enhanced spontaneous locomotor activity and a reduction in the apomorphine-induced increase in locomotion four to nine days after cessation of treatment. However, nine

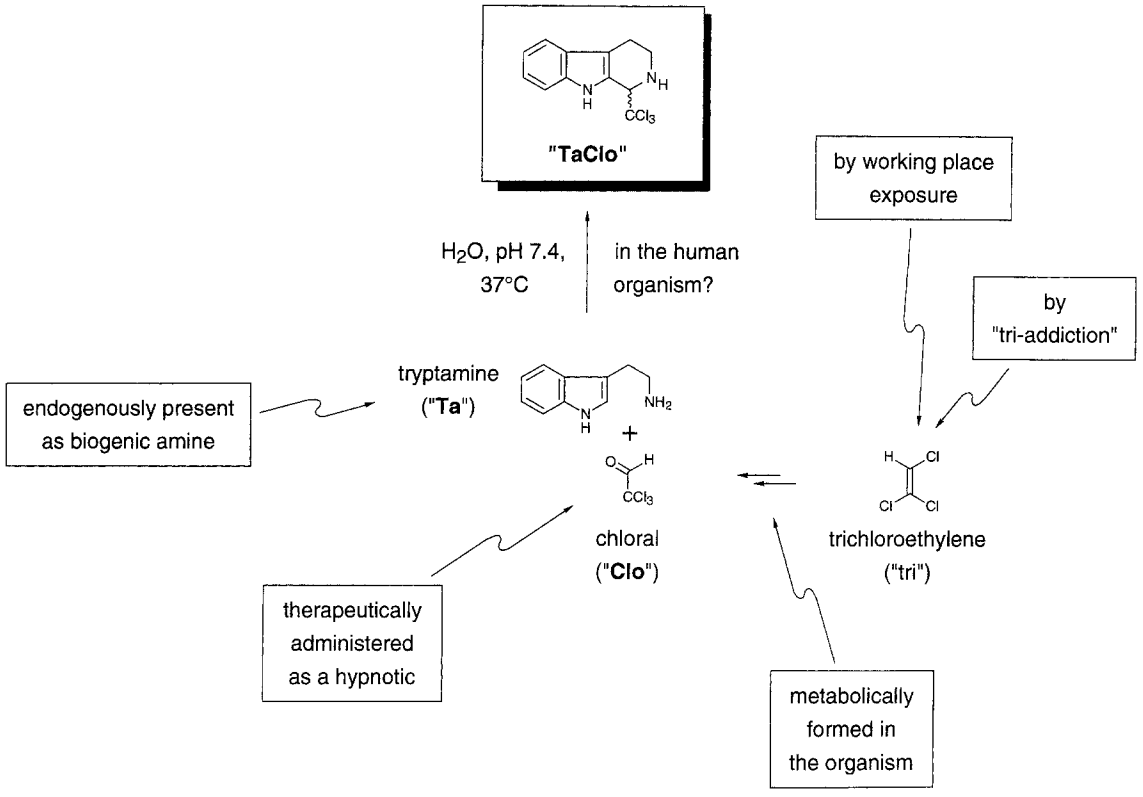



**Fig. 3.** Formation of TaClo in the human organisms (according to Bringmann et al., 1995). *TaClo* 1-trichloromethyl-1,2,3,4-tetrahydro-β-carboline

weeks following treatment animals walked more slowly during 12 hours of nocturnal activity, and apomorphine-induced locomotion was decreased 12 weeks later. These results suggest that exposure to TaClo may exert a progressive neurotoxic effect on the dopaminergic nigro-striatal system. The damage to the dopaminergic system can also be demonstrated in vivo by a pulsed voltammetric procedure (Grote et al., 1995). Using this procedure, pre-synaptically liberated dopamine is indirectly measured via the DOPAC-signal. DOPAC is formed intracellularly from dopamine by an MAO-catalysed oxidation and therefore acts as a marker for the presynaptically liberated dopamine (Zetterström et al., 1988). The unilateral nigral injection of either TaClo (10μg) or the N-methylated derivative (10μg) evokes a diminished DOPAC-signal in the ipsilateral striatum: During the first week following the lesion there is a 55 and 90% reduction, respectively, on the side of the injection compared to the contralateral, intact side, while three weeks later the signals are diminished by 74 or 93 (Grote et al., 1995). Again, these results confirmed the progressive course provoked by exposure to TaClo. In addition they show that the N-methylated TaClo is more toxic than TaClo.

*2.5.2.2 The mechanism of the neurotoxic action.* Because analogues of MPTP and $MPP^+$ lacking a N-methyl group are essentially devoid of toxicity (see

Testa et al., 1985), a critical step in the activation of β-carbolines should be a reaction of N-methylation. Numerous endogenous compounds undergo methylation of an endocyclic or exocyclic nitrogen atom by such enzymes as phenylethanolamine N-methyltransferase, histamine N-methyltransferase, or indolethylamine N-methyltransferase. Xenobiotics undergoing N-methylation in brain and/or other tissues by these or other enzymes include theophylline, normorphine and pyridine, among a number of other heterocyclic compounds (see Testa et al., 1985). Indeed, Fields et al. (1992) have also reported that selected β-carbolines are N-methylated by preparations of mammalian brain. Using rat striatal synaptosomal preparations possible β-carboline substrates of the synaptic dopamine transporter have been screened (Drucker et al., 1990). The partially competitive nature of inhibition by one of the more effective N-methylated compound, 2-methyl-harmine, was consistent with uptake of those by the synaptosomal dopamine uptake system. Their oxidation products, the β-carbolinium, have been shown to inhibit $NAD^+$-linked $O_2$ consumption in rat liver mitochondria: Two derivatives of 2-methylharmine, the O-demethylated 2-methylharmol ($IC_{50}$ 209 μM) and the 2-methylharmine ($IC_{50}$ 186 μM), were approximately equipotent with $MPP^+$ ($IC_{50}$ 171 μM), a potent inhibitor of complex I (Albores et al., 1990). Moreover, using rat brain homogenates and submitochondrial particles it has been recently demonstrated that TaClo specifically inhibits the electron transfer from complex I towards ubiquinone in a concentration (800 μM) 10-times lower than that of $MPP^+$ (Janetzky et al., 1995). By extending the preincubation time from five to 30 min complex I is already inhibited by 400 μM TaClo. The N-methylated TaClo derivative demonstrate an even greater inhibitory effect on complex I (total inhibition at a concentration of 250 μM). In addition to inhibition of complex I complex II was totally blocked at the same concentration (Janetzky et al., 1995). Thus, respiratory inhibition may underlie the neurotoxicity of β-carbolines observed in primary cell cultures of C57/Black mouse mesencephalon containing dopaminergic neurons (Rausch et al., 1995) and in vivo (Neafsey et al., 1989; Grote et al., 1995; Sontag et al., 1995). However, Krueger et al. (1993) could only detect partial inhibition of complex I and II activities at very high concentrations of the β-carbolinium compounds, 2,9 dimethylharmaninium and 2,9-dimethylnorharmaninium.

## 2.6 Intranigral injections of iron (III)

### 2.6.1 Neurotoxic effects

*2.6.1.1 Behavioural changes.* The unilateral injection of 50 μg of iron(III) into the SN of the rat produces strongly altered motor behaviour in the animals, which even three weeks after application is expressed by a reduction in spontaneous locomotor activity in a strange environment, by a lower frequency of getting up onto the hind-legs, by a transient appearance of "freezing" phenomena and by spontaneous ipsilateral rotations (Ben-Shachar and Youdim, 1991); these ipsilateral rotations are accentuated by amphetamine.

*2.6.1.2 Histopathological and neurochemical changes.* The unilateral nigral injection of 50 μg iron(III) evokes an average 95% reduction of the dopamine concentration in the ipsilateral striatum, as well as smaller reductions in the dopamine metabolites DOPAC and HVA (85% or 45% respectively) (Ben-Shachar and Youdim, 1991). Similar changes are also observed at lower iron(III) concentrations (70–350 ng) (Sengstock et al., 1992, 1993). Other neurochemical striatal markers, such as noradrenaline, serotonin and 5-hydroxyindoleacetic acid are on the other hand unchanged (Ben-Shachar and Youdim, 1991; Arendash et al., 1993).

Histopathological investigations predominantly show damage of the SN pars compacta (Sengstock et al., 1992; Arendash et al., 1993). As early as 24 hours after intranigral application of 70 ng of iron(III) iron-stained astrocytes and microglia can be demonstrated. Immediately adjacent to these iron-stained cells there is a severe loss of neurons and a reactive gliosis, still demonstrable after at least six months, as well as a decreased number of TH-immunoreactive neurons.

This damage to the dopaminergic system produced by intranigral iron injection can also be demonstrated in vivo by a pulsed voltammetric procedure (Wesemann et al., 1993, 1994). In the striatum of rats that have been lesioned with iron(III) (50 μg) the DOPAC-signal is found to be diminished. The time course of this signal is interesting (Wesemann et al., 1994): during the first week following the lesion there is a 79% reduction on the side of the injection compared to the opposite side, while three to six weeks later the signals are diminished by 86 or 97%. This is the first time that a toxic insult has been shown to result in a chronic and progressive course in an animal model. This chronic and progressive course provoked by the nigral iron(III) injection is confirmed by other parameters (Sengstock et al., 1994): first, there is likewise a progressive course in the effect on dopamine and HVA in the striatum, as determined at post mortem; second, a progressive atropy of the SN has been found; and third, there is progressive alteration in the apomorphine-elicited rotational behaviour.

### 2.6.2 The mechanism of the neurotoxic action

The mechanism of the neurotoxic effect of intra-nigrally injected iron(III) has not been extensively investigated and is only partly understood. It is assumed that iron(III) is taken up into the neurons and glia by transferrin-receptors (Arendash et al., 1993). It is probably reduced to iron(II) in the cytosol of these cells. Iron(II) shows a cytotoxic action in primary cultures of neurons derived from the mesencephalon of rat embryos (Michel et al., 1992). In a reaction similar to the Fenton reaction, excess iron(II) could catalyse the formation of hydroxyl free radicals from hydrogen peroxide which is an endogenous product of enzymatic dopamine metabolism. This proposed mechanism for the neurotoxic effect is indirectly confirmed by the demonstration of increased amounts of thiobarbituric acid-reactive compounds (a measure of lipid peroxidation) (Arendash et al., 1993; Wesemann et al., 1993).

### *3. Pharmacological- and neurotoxin-induced models of tremor*

Resting tremor is an essential diagnostic criterion of PD (Birkmayer and Riederer, 1985). Although the symptoms of this tremor can be treated using a variety of different strategies, such as anticholinergic drugs, L-DOPA or dopamine-receptor agonists, as well as by stereotactic disconnection of the ventrolateral thalamic nucleus, there is still only a limited understanding of the pathophysiological, neurochemical and neuropathological origins of the tremor. A study published recently showed that patients with PD of an akinetic-rigid type of progression exhibited a greater loss of neurons in the locus ceruleus of the lateral SN than those in whom the progression was of a tremor-dominant type (Paulus and Jellinger, 1991). Furthermore, more severe structural alterations could be observed, such as gliosis, extraneuronal melanin-deposits and neuroaxonal dystrophy in the SN.

#### 3.1 Oxotremorine tremor

Experimental animal models of tremor have predominantly been applied to the investigation and development of strategies for treating the symptoms. In 1956 Everett discovered the tremorigenic effect of tremorine, of which the active metabolite is oxotremorine. The effects of oxotremorine, particularly in the mouse, are to produce tremor; but hypothermia, rigidity and a range of parasympathomimetic symptoms such as for example hypersalivation, are also elicited. Oxotremorine is a selective agonist of the muscarinic acetylcholine receptor, and systemic application of oxotremorine stimulates acetylcholine receptors both in the periphery and also in the CNS. The experimentally-induced tremor in the animals results from the stimulation of muscarinic acetylcholine receptors in the basal ganglia, because intrastriatal injection of oxotremorine provokes tremor in most species of animals (Haefely, 1978).

According to investigations by Jurna et al. (1970, 1973) the oxotremorine-tremor depends on rhythmic discharges of deinhibited $\gamma$-motoneurons super-imposed on a baseline of $\alpha$-motoneuron activity. Quaternary anticholinergic drugs which do not pass the blood-brain-barrier inhibit the peripheral parasympathetic action of oxotremorine, but not the centrally-mediated symptoms of tremor and hypothermia. The oxotremorine model is thus neither a genuine model of PD nor one suitable for the discovery of new anti-tremorigenic substances, but only picks up centrally acting antagonists of the muscarinic acetylcholine-receptor (Haefely, 1978). Anti-Parkinson drugs that act by affecting dopaminergic mechanisms, only partially influence the oxotremorine tremor: an anti-tremorigenic effect, one that may possibly be elicited via peripheral mechanisms, is only observed at high dose levels (Horst et al., 1973).

#### 3.2 The "sinistrotorsional" model

The injection of cholinesterase inhibitors able to penetrate into the brain, into the right carotid aorta of guineapigs leads to circling movements of short

duration and a flexing of the longitudinal axis of the body to the left ("sinistrotorsion") (for a review see Haefely, 1978). This phenomenon depends on a brief cholinergic stimulation of the right-hand brainstem. The extent to which the vestibular nuclei, the SN and other structures may be primarily involved is not clear. The "sinistrotorsion" may possibly be dependent on activation of dopaminergic nigro-striatal neurons (Haefely, 1978). Anticholinergic anti-Parkinson drugs, but also other centrally active drugs with antimuscarinic components to their activity, such as tricyclic antidepressants for example, are able to prevent the "sinistrotorsion" phenomenon. However, L-DOPA, even at the highest possible doses is ineffective (Haefely, 1978).

### 3.3 Tremor in the monkey MPTP model

As already described in a previous chapter, tremor is not seen in the MPTP model in the mouse. In the MPTP model in monkeys, resting tremor is only encountered infrequently (for a review see Stern, 1990; Gerlach et al., 1991). In 50 MPTP-treated macaques only a single case was observed to show a resting tremor (Gomez-Mancilla et al., 1991). This was investigated and characterized using electromyography (EMG), and showed not only a rhythmic frequency (7–8 Hz) but also successive contractions of antagonistically acting muscles of a differential frequency. The literature also contains references that tremor occurs in MPTP-treated monkeys if one activates the animals by means of external stimuli (Burns et al., 1983), but their appearance is more like a postural or action tremor.

## The relevance of the experimental models to PD

The reserpine model was the first model that became available for testing symptomatic anti-Parkinson treatments. The partial removal of the reserpine-syndrome in the rat (akinesia, bradykinesia, hypokinesia, catalepsy, tremor) by L-DOPA led to the development of the L-DOPA therapy, which has remained the cornerstone of anti-Parkinson treatment up to the present. Although the detailed mechanism of action of the reserpine model is not completely known, in essence it is explained on the basis that what is mainly involved is a reversible functional reduction of dopaminergic neurotransmission, following a diminished capacity of the storage vesicles for storing dopamine. A substantial drawback of this model is that reserpine has a non-specific effect on all the monoaminergic neurotransmitters. Pharmacologically-induced functional disturbances of dopaminergic neurotransmission can also be provoked by neuroleptics, such as haloperidol for example (Table 7). Furthermore, dopamine-D2-receptor-deficient mice exhibit a Parkinsonian-like locomotor impairment that broadly resemble neuroleptic treatment (Baik et al., 1995), indicating that the observed effect by dopamine antagonist treatment is due to the specific blocking of D2 receptors. In all these models akinetic symptoms may be produced to a greater or lesser extent, but the significance of these species-specific functional disturbances for the pathophysiology of the fully-developed Parkinsonian symptomatology (akinesia,

**Table 7.** Relevance of the animal experimental models for Parkinson's disease

| Animal model | Symptoms | Histopathology | Pathobiochemistry | Etiological significance | Disadvantages |
|---|---|---|---|---|---|
| *Pharmacologically-induced functional disturbances of dopaminergic neurotransmission* | | | | | |
| Reserpine model of mice and rats | Bradykinesia, hypokinesia, catalepsy, tremor? | | | None | Nonspecific liberation of neurotransmitters |
| Neuroleptic-induced catalepsy in rats | Akinesia, muscular rigidity, tremor of the extremities | | | None | Nonspecific, all the dopaminergic systems are affected |
| Haloperidol-induced akinesia and bradykinesia in mice | Akinesia, bradykinesia | | | None | Nonspecific, all the dopaminergic systems are affected |
| *Experimentally-induced degeneration of nigro-striatal dopaminergic neurons* | | | | | |
| Unilateral 6-hydroxydopamine model in the rat | Characteristic circling behaviour after apomorphine | Degeneration of dopaminergic neurons in the SN | Loss of dopamine in the striatum | Oxidative stress | Acute model, intracerebral injection, "hemiparkinson" model |
| Methamphetamine model of mice and rats | Not known | Reduction in TH-immunoreactive neurons in the SN | Loss of dopamine in the striatum | Oxidative stress | Acute model, no systematic histological investigation |
| Iron(III) model in the rat | Akinesia, freezing-phenomenon, characteristic circling behaviour | Atrophy of the SN, gliosis, fewer TH-immunoreactive neurons in the SN | Loss of dopamine in the striatum | Oxidative stress | Intracerebral injection, effect in other animal families and species not investigated, specificity? |
| MPTP model of the mouse | Hypokinesia? | Reduction in TH-immunoreactive neurons in the SN and VTA | Loss of dopamine in the striatum and limbic regions | Endogenous or exogenous neurotoxins, oxidative stress, inhibits complex I | Not a permanent model, not progressive |
| MPTP model of the monkey | Akinesia, rigidity, tremor | Reduction in TH-immunoreactive neurons in the SN and VTA | Loss of dopamine in the striatum and limbic regions | Endogenous or exogenous neurotoxins, oxidative stress, inhibits complex I | Not progressive, tremor not always observed |

*SN* substantia nigra, *TH* tyrosine hydroxylase, *VTA* ventral tegmental area

rigidity, tremor) is difficult to assess, because of the differences in the anatomy and function of the CNS between primates and rodents. All those models which produce a pharmacologically-induced functional impairment of dopaminergic neurotransmission are nowadays only rarely used, and if at all, then mainly as screening methods for testing methods of symptomatic treatment. They are hardly suitable for dissecting out causal mechanisms.

The 6-OHDA model in the rat, in which there is a unilateral degeneration of dopaminergic nigro-striatal neurons with a depletion of dopamine in the striatum, is nowadays still a widely used approach to the development of strategies for the symptomatic treatment of PD, but also to some degree for the elucidation of the disease mechanism, bearing in mind the participation of "oxidative stress" in its pathogenesis (Table 7). This model suffers above all from two drawbacks. First, the neurotoxin has to be applied stereotactically to the appropriate brain regions, and its action seems to be most effective when it is given into the striatum. Second, although one can classify dopamimetically acting drugs into direct and indirect agonists on the basis of their rotatory effects, it is not so easy to distinguish drugs acting on other neurotransmitter receptors. What makes it worse is that his characteristic rotational behaviour, which is quantified and used as a measure of the effective potency of the substance being tested, is not relevant to the pathophysiology of the symptoms of PD.

Although the methamphetamine model in the mouse has not yet been thoroughly and systematically investigated, systemically applied methamphetamine does appear specifically to induce the essential histopathological and pathobiochemical changes characteristic of PD (Table 7). The neurotoxic effect does not however appear to affect the animals' spontaneous motor behaviour.

Nowadays the most widely used and best investigated model is the MPTP model. The partial explanation of the neurotoxic mode of action has contributed to the development of views on the decisive processes relating to the death of nerve cells, and has therefore made important contributions to our understanding of the pathogenesis of PD. The MPTP model is even nowadays still a valuable model for testing neuroprotective strategies. Both in mice and in non-human primates it is capable of simulating the most significant features of the histopathological and pathobiochemical changes in PD (Table 7). Although tremor is the one symptom which is not observed very often, a fully developed model of PD can be provoked with MPTP in some individual monkeys. From a histopathological viewpoint, one can observe the same loss of dopaminergic neurons in the VTA and of noradrenergic neurons in the locus ceruleus so characteristic of the human PD, particularly in older monkeys. These injuries lead to the characteristic changes of the neurotransmitters dopamine and noradrenaline in the brains of the animals, but neuropeptides remain unaltered.

The ability of MPTP to give rise a parkinsonian condition has led to suggestions that there may be a naturally occurring or environmental toxin that causes PD. Potential candidates that have been shown to be neurotoxic in animals are some endogenously formed tetrahydroisoquinolines (e.g. N-

methyl-salsolinol) and β-carbolines (e.g. 2-methyl-norharman, TaClo). However, the implications for the etiopathogenesis of PD is unknown. Several lines of evidence suggest the possible involvement of an environmental factor in the development of PD, but the data are confusing and often apparently contradictory (e.g. Tanner, 1989). If the idiopathic disease were to result from a longer term exposure to an environmental or endogenous formed toxin, a compound as toxic as MPTP would be an unlikely candidate for a causative factor unless it was present at extremely low concentrations. Although, tetrahydroisoquinoline and its 1-methyl-derivative have been found in low concentrations in human caudate nucleus and frontal lobe (0.01–1.68 and 0.01–2.00 ng/g brain, respectively), there were no significant differences between its concentrations in normal and parkinsonian brains (Ohta et al., 1987). Slightly lower but significant concentrations of salsolinol and of N-methyl-salsolinol were found in the CSF of parkinsonian de novo patients, untreated with L-DOPA, compared to those of controls (Dordain et al., 1984). However, higher levels of these compounds have been detected in several brain regions, including the caudate nucleus, the putamen, the hippocampus, and the cortex, of intoxicated alcoholics (Sjöquist et al., 1982).

All these models, where an experimentally-induced destruction of nigro-striatal neurons is produced, have this in common that the neurotoxic process shows no progression; in other words when the neurotoxin is no longer applied there is no further chronic continuation of the neurotoxic process independent of the presence of the neurotoxin. A chronic progressive course is solely observed in the "iron" and "TaClo model". Since PD develops gradually over a long period until the typical symptoms come to their full expression (5–15 years), a question mark hangs over the relevance of these mostly acute models to the pathogenesis of the human disease. The neurotoxic effects of the repeated application of small doses of 6-OHDA, methamphetamine, iron(III) and MPTP in rodents over a long period have not been investigated. In non-human primates long-term (up to 10 month) application of smaller doses of MPTP (0.25–2.5 mg/kg i.p. twice a week) do elicit permanent damage to the nigro-striatal dopaminergic system, nevertheless MPTP-treated marmosets recover as far as their motor behaviour is concerned, possibly as a result of compensatory and/or regenerative processes (Ricaurte et al., 1986; Pérez-Otaño et al., 1991; Russ et al., 1991; Colosimo et al., 1992).

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