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Neurochemical findings in the MPTP model of Parkinson's disease

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Summary. Animal models are a very important approach to study the pathogenesis and therapeutic intervention strategies of human diseases. Since many human disorders do not arise spontaneously in animals, characteristic functional changes have to be mimicked by neurotoxic agents. For instance, the application of the dopaminergic neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is able to produce striking similarities to Parkinson's disease (PD) diagnosed in humans. MPTP is thought to selectively damage dopaminergic neurons predominantly those originating in the substantia nigra pars compacta (SNc) which leads to impaired dopaminergic neurotransmission accompanied by a loss of dopaminergic nerve terminals in the striatum. MPTP-induced neurochemical, behavioral, and histopathological alterations replicate very closely the clinical symptoms of PD patients, which will be discussed in this paper and render the MPTP model currently the most favored PD model to study therapeutic intervention strategies in an easy and reliable way in preclinical studies.

We and many other research groups propose that the knowledge about the neurotoxic mechanisms of MPTP such as mitochondrial dysfunction with breakdown of energy metabolism and free radical production will help us to understand the underlying mechanisms of PD, which are not fully understood yet. In particular, the novel aspects of inflammatory processes and the involvement of reactive nitrogen species in addition to reactive oxygen species seem to be important milestones for a better understanding of the neurodegenerative effects of MPTP.

In this review we focus on the MPTP mouse model which is easy practicable and widely used in neuroscience research and draw comparisons to the human pathology in PD.

Keywords: Animal models, MPTP, neurodegeneration, neurotoxicity, Parkinson's disease.

Introduction

Parkinson's disease (PD) is the most frequent neurological disorder of the basal ganglia. The French neurologist Charcot named this most prominent movement disorder by the London physician James Parkinson, who was the first to describe the motor symptoms in 1817 in the "Essay on the Shaking Palsy".

The disease is characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) leading to a dopamine (DA) depletion in the striatum (Ehringer and Hornykiewicz, 1960; Riederer and Wuketich, 1976; Hornykiewicz and Kish, 1986). Moreover, the impairment in the dopaminergic neurotransmission leads to profound functional changes in the input and output structures of the basal ganglia (McGeer et al., 1987; Bergman et al., 1990; Sian et al., 1999). As a result parkinsonian patients loose the ability to control voluntary movements and exhibit tremor, muscular rigidity, akinesia and bradykinesia with difficulties in writing, walking, speaking, masking of facial expression, slowness in initiating and executing movements as well as stooped posture and instability (Fahn, 1988).

In the early 1980s the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Fig. 1) was discovered accidentally. As a concomitant of a meperidine analogue 1-methyl-4-phenyl-propion-oxypiperidine (MPPP) used as "synthetic heroin" MPTP produced a parkinsonian syndrome in young drug abusers after its unintentional self-administration (Davis et al., 1979; Langston et al., 1983).

Most of the biochemical, pathological and clinical features induced in these young addicts correspond to the hallmarks of PD, with the exception of the presence of typical Lewy bodies (Langston et al., 1983). A recently published follow up study presented evidence for the severe and unremitting parkinsonism in these patients probably caused by an active nerve cell degeneration induced by MPTP with characteristic features of neuroinflammation (Langston et al., 1999).

Injected in primates MPTP seems to produce nearly the same pathobiochemical changes as observed in PD patients (Burns et al., 1983; Cohen et al., 1984; Jenner et al., 1986). Therefore, MPTP was used to develop animal models for elucidating the cellular mechanisms of the degenerative

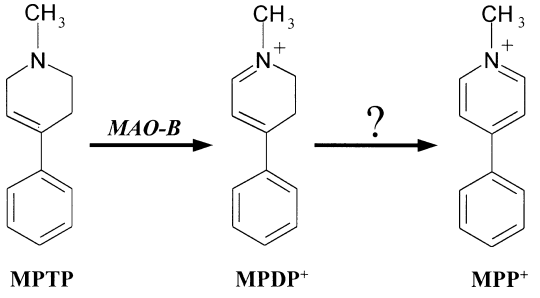

Fig. 1. Biotoxification of MPTP: in a two-step biotransformation MPTP is at first converted to $MPDP^+$ via MAO-B. $MPDP^+$ is spontaneously oxidized to form MPP^+ by a mechanism which is not fully understood yet

processes in PD (for review see Tipton and Singer, 1993; Przedborski and Jackson-Lewis, 1998) and for testing new therapeutic strategies (Heikkila et al., 1984; Schmidt and Ferger, 2001; Teismann and Ferger, 2001). It is postulated that MPTP may also become a leading substance for research of environmental toxins, which may initiate or promote PD. Epidemiological data suggest that environmental factors are able to produce PD in individuals over 50 years of age (Tanner et al., 1999). Theoretically, the exposition with a low dose of a MPTP-like toxin may occur several years before the first symptoms become manifest. This may explain that only few people of the 300 individuals who may have been exposed to MPTP suffered from PD (Ballard et al., 1985). However, up to date there is no experimental evidence for MPTP-like toxins in the SNc in PD, because neither MPTP nor related compounds could be determined in the brain or CSF of PD patients so far (Ikeda et al., 1992; Goodwin and Kite, 1998). Nevertheless, the MPTP induced neurochemical, neuroanatomical and behavioral alterations are invaluable to study the pathological mechanism of PD in animal models.

In this review we would like to point out that although the mechanism of MPTP toxicity has already been described by the pioneering work of several scientists in the mid 80s the application of MPTP using different treatment schedules and the new findings which were obtained using genetically modified mice as well as sophisticated bioanalytical techniques promise a respectable progress for experiments in future. In particular, the novel aspects of inflammatory processes, cytokines and the involvement of reactive nitrogen species in addition to reactive oxygen species in MPTP toxicity is worth mentioning. All this predestinates MPTP to probe novel therapeutic strategies in PD in a better way than ever before. However, we also like to emphasize that not only one MPTP model but instead many MPTP models exist which critically depend on different factors mentioned in the following paragraphs.

Species differences in MPTP neurotoxicity

The discovery of MPTP paved the avenue for a model of PD, which is stated to be the best one up to date (Beal, 2001). The MPTP model is mainly used in non human primates and mice but also in other species such as dogs, cats, sheep and goldfishes (Gerlach and Riederer, 1996). In contrast to primates rodents are less sensitive to MPTP toxicity. However, using an appropriate application schedule of MPTP (please see below), the C57black/6 strain was found to be sensitive to a systemic injection of MPTP and more selective in terms of targeting the nigrostriatal dopaminergic neurons than other mice strains. The authors think that the MPTP mouse model seems to be the most practicable choice to study neuroanatomical and neurochemical alterations. Obviously the best choice for behavioral analysis is the MPTP monkey model. At first glance the behavioral changes in mice tend to recover completely, but we expect that the use of more sophisticated behavioral tests and MPTP treatment schedules together with enhancers of MPTP toxicity such as diethyldithiocarbamate, acetaldehyde and probenecid will overcome the difficulties in testing mice behavior more reliably in future. Besides the strain the

age of the animals at the time point of MPTP administration plays a pivotal role in terms of sensitivity against MPTP-induced neurodegeneration. Aged animals show severe and long lasting lesions. In contrast, young animals require a higher dose to reach a comparable lesion (Gerlach et al., 1991). There is also a debate of the reversibility of MPTP-induced degeneration. Repeated administration produces a more robust and irreversible degeneration than a single administration of MPTP. We found that the MPTP-induced degeneration tends to be partly reversible indicated by a partial recovery of MPTP-induced striatal dopamine depletion (please see paragraph Neurochemistry and immunohistochemistry, Fig. 6). In young mice the ability of repair and compensatory mechanisms seems to be more efficient than in aged mice where recovery is comparably lower (Ricaurte et al., 1987).

Definitively, rats are not recommended for MPTP research, because systemic injection of MPTP in rats failed to produce parkinsonism as obtained in mice (Heikkila et al., 1984; Boyce et al., 1984; Sahgal et al., 1984). The resistance of rats to MPTP toxicity may be related to the different metabolism of MPTP and sequestration of MPP^+ by rats in comparison to mice and monkeys.

Schedules of MPTP administration in mice

Remarkably, the schedule of MPTP administration does not only substantially influence the time course of neurodegeneration but may also provide a better understanding of the distinct phases of neuronal death such as the presymptomatic, the immediate onset, the progressive and the final stage of PD in humans.

Summarizing selected reports from the literature one may classify at least 4 different types of MPTP models, which are briefly presented and annotated in the following.

A: Model for presymptomatic PD: suitable for the study of compensatory mechanisms

Acute treatment with a low dose of MPTP (1×10–20 mg/kg) (Aubin et al., 1998).

B: Immediate onset PD model for rapid degeneration with necrotic cell death

Acute treatment with an intermediate dose of MPTP (4×20 mg/kg, 2 h apart) (Jackson-Lewis et al., 1995).

C: Subchronic PD model for delayed degeneration with apoptotic cell death

Subchronic treatment: 1–2 daily injections of MPTP (20–30 mg/kg, 5 days) (Tatton and Kish, 1997; Vila et al., 2000).

D: Progressive chronic PD model (preferential counterpart of idiopathic PD)

Chronic treatment: one daily injection of MPTP (4 mg/kg, 20 days) (Bezard et al., 1997a,b).

However, one should keep in mind that all mentioned models use end points in the range of days or weeks and are far away from the slowly progressing neurodegenerative processes of PD in humans which develop over decades.

Mechanisms of MPTP toxicity

Biotransformation

MPTP itself is not toxic in the brain. It rather requires a two-step biotransformation process to form the metabolite MPP^+ (see Fig. 1), which is the ultimative neurotoxin. In contrast to the charged MPP^+, which cannot cross the blood brain barrier, the lipophilic MPTP rapidly enters the brain after systemic administration. In a first step, MPTP is converted via monoamine oxidase B (MAO-B) to form the 1-methyl-4-phenyl-1,2-dihydroxypyridinium ion ($MPDP^+$) (Chiba et al., 1984; Markey et al., 1984). Interestingly, MAO-B is mainly present in astrocytes and also in serotonergic neurons (Takada et al., 1990; Di Monte et al., 1991) but not in dopaminergic neurons itself (Westlund et al., 1985). In a second step $MPDP^+$ is spontaneously oxidized to MPP^+. Theoretically, both isoforms, namely MAO-A and B, are able to convert MPTP. However, MAO-B is more likely to be the key enzyme than MAO-A. Thus, inhibition of MPTP metabolism using MAO-B inhibitors, but not MAO-A inhibitors, effectively prevented MPTP neurotoxicity (Heikkila et al., 1984; Cohen et al., 1984). Figure 2A represents the clear reduction of striatal MPP^+ levels after pretreatment with the MAO-B inhibitor selegiline (deprenyl), which again clearly indicates that MAO-B inhibition is able to prevent MPP^+ formation. Furthermore, animals with high MAO-B activity in the endothelium of brain capillaries of the blood brain barrier, such as rats, are probably resistant to MPTP, because MPTP uptake into their brains is prevented by rapid metabolism.

MPP^+ is released by glial cells into the extracellular space by an active mechanism using the extraneuronal monoamine transporter which was studied in detail by Russ and coworkers (Russ et al., 1996). Subsequently, MPP^+ can be specifically taken up into dopaminergic nerve terminals via the plasma membrane dopamine transporter (DAT) (Chiba et al., 1985; Javitch et al., 1985). Recently it was demonstrated that mice with a deficiency of the DAT are resistant against MPTP toxicity (Gainetdinov et al., 1997; Takahashi et al., 1997). Overexpression of DAT may result in enhanced MPTP neurotoxicity. The DAT is not only expressed at the terminal side but also on dendrites in the cell body region, which offers the possibility that MPTP toxicity is targeting both, the striatum and the substantia nigra.

Intraneuronally MPP^+ is taken up by the vesicular monoamine transporters (VMATs) and sequestrated into synaptosomal vesicles or it accumulates in mitochondria via energy-driven uptake (Singer et al., 1987). The intravesicular storage of MPP^+ via VMAT suppressed its toxicity in vitro (Liu et al., 1992). This was confirmed in vivo using VMAT-2 deficient mice which consequently demonstrated an increased MPTP toxicity (Takahashi et al., 1997; Gainetdinov et al., 1998).

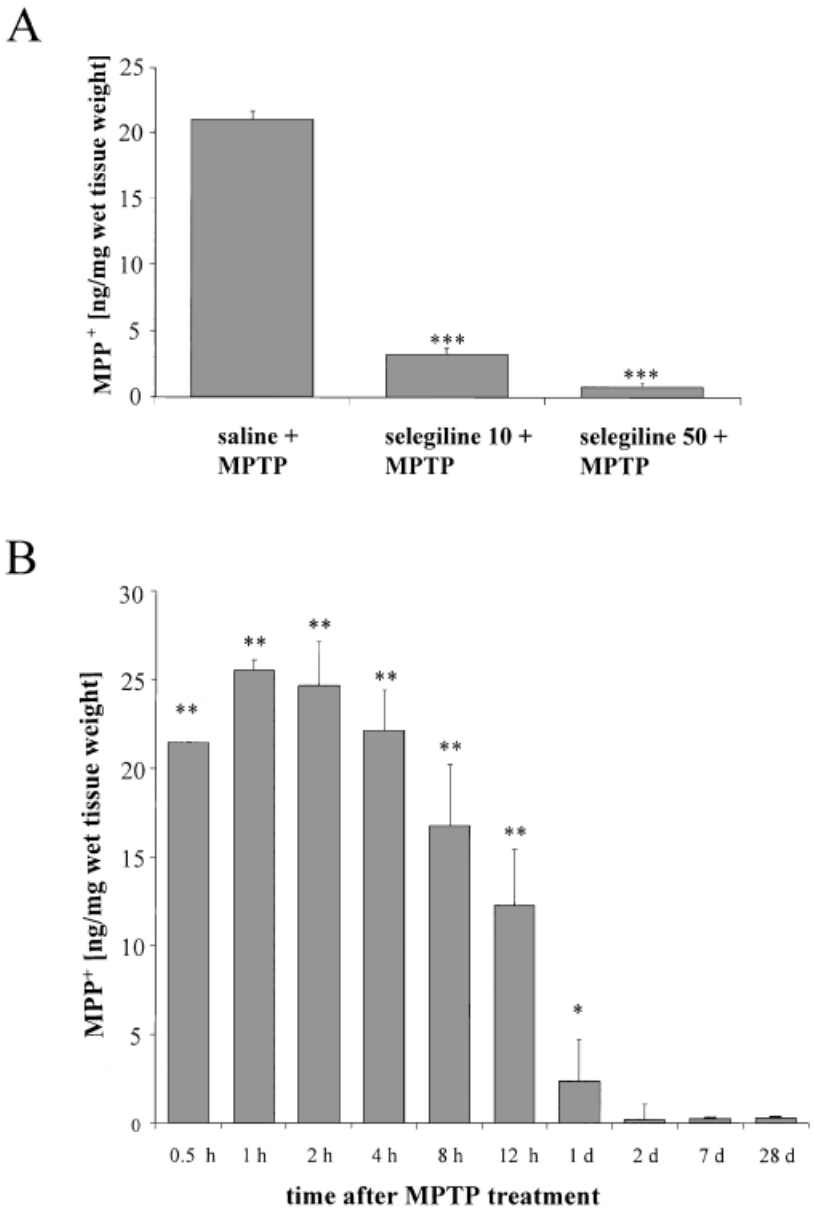

Fig. 2. Effects of the MAO-B inhibitor selegiline (10 and 50 mg/kg i.p.) on striatal MPP^+ levels in mice (**A**). MPP^+ levels were measured 2 h after MPTP treatment (30 mg/kg s.c.) using HPLC in combination with UV detection. Values are given as mean ±SEM of n = 5–6 mice. ANOVA with subsequent Bonferroni test for comparison with the saline +MPTP group (***$P < 0.001$). Time-course of MPP^+ formation after MPTP treatment (40 mg/kg i.p.) in mice (**B**). Striatal MPP^+ levels reached a maximum (25 ng/mg wet tissue weight) 1 hour after MPTP injection. After 2 days no significant increase was detectable anymore. Data are mean ±SEM of n = 5–6 mice. Statistical analysis was performed using Kruskal Wallis H-test with subsequent Mann Whitney U-test for comparison with the control group (*$P < 0.05$, **$P < 0.01$) (Schmidt and Ferger, unpublished data, for a detailed description of tissue preparation and HPLC analysis of MPP^+ please see Teismann and Ferger, 2001)

Mitochondrial impairment

Accumulation of MPP^+ in the mitochondria of dopaminergic neurons leads to an impairment in mitochondrial function. MPP^+ binds to complex I of the respiratory chain (Nicklas et al., 1985; Ramsay et al., 1991), which blocks the electron transport, and thus leads to an energy failure with ATP depletion and an increase in the formation of free radicals. The time course of rapid ATP loss and restoration (Chan et al., 1991, 1992) is correlated with MPP^+ brain levels (Fig. 2B).

Energy failure

Intact mitochondrial function is necessary for cellular energy supply. MPP^+ inhibits complex I and impairs ATP formation which in term disables or reduces energy-dependent processes such as maintenance of the calcium homeostasis and of the cellular membrane potential as well as ion- and transmitter transport in general (Di Monte, 1991; Royland and Langston, 1998). The energy failure due to MPTP-induced ATP depletion is aggravated by secondary steps due to energy consuming repair processes. In particular, enzymes such as poly(ADP-ribose)polymerase (PARP) which require ATP for DNA repair are critically involved in MPTP toxicity. This line of evidence was investigated with pharmacological studies using PARP inhibitors (Cosi et al., 1996) or PARP (−/−) knockout mice which both showed protection against MPTP toxicity.

Additionally, MPP^+ is thought to inhibit the α-ketoglutarate dehydrogenase of the tricarboxyclic acid cycle (complex II of the respiratory chain) (Mizuno et al., 1987). This mechanism acts synergistically to enhance the MPTP-induced disruption of cellular energy metabolism.

Calcium homeostasis

An apparent consequence of severe energy impairment is the decreased activity of the energy dependent calcium-ATPase which leads to intraneuronal calcium-overload. Elevated intracellular calcium levels activate degradative enzymes like phosphatases and proteases. Degradation of cell membranes or the cytoskeleton in term results in disrupted cell function, loss of cell membrane potential and finally neuronal death. Endogenous mechanisms which are able to bind the excess of calcium or exogenously applied calcium channel blockers showed protection against MPTP-induced nigral degeneration (German et al., 1992; Kupsch et al., 1995, 1996).

Glutamate release

Excessive MPP^+ concentrations may promote excitotoxicity, as local administration of MPP^+ via reverse microdialysis enhanced glutamate release (Carboni et al., 1990). Besides its physiological role as the most abundant excitatory amino acid in neurotransmission, glutamate exerts neurotoxic properties in a dual way. Direct excitotoxicity caused by excessive glutamate release and activation of NMDA receptors leads to a massive influx of calcium

and thereby induces the formation of reactice oxygen species (ROS) as well as to a reduced intracellular glutathion synthesis (Murphy et al., 1989). Indirect excitotoxicity is postulated to be based on impaired mitochondrial function and a cascade of events which enable normally "non-toxic levels" or lower levels of glutamate to become cytotoxic (Beal et al., 1993).

Reactive oxygen and nitrogen species

ROS are formed continuously in the body as byproducts of numerous biochemical reactions. Compared to other brain regions the SNc is exposed to higher levels of oxidative stress caused by catabolism of dopamine via MAO-B-mediated deamination, dopamine autoxidation and high levels of iron which together induce a high degree of ROS formation (Coyle and Puttfarcken, 1993). In addition, low glutathion peroxidase levels diminish the ability of the SNc to cope with oxidative stress (Sian et al., 1994).

MPP^+ is known to cause striatal dopamine efflux measured with in vivo microdialysis in the extracellular space (Lang et al., 1990; Santiago et al., 1991). Furthermore, MPTP administration produces an increased dopamine turnover (Teismann and Ferger, 2001) which resembles the enhanced dopamine turnover in PD. Indeed, in the later stage of PD when already more than 80% of the dopaminergic neurons underwent neurodegeneration the remaining dopaminergic neurons try to compensate for the reduced number of neurons by producing more dopamine (Fahn and Cohen, 1992). From dopamine oxidation one molecule H_2O_2 per molecule dopamine is generated and reacts with Fe^{2+} ions to form ROS by the Fenton reaction.

Among ROS superoxide is less deleterious compared with hydroxyl radicals. Hydroxyl radicals are the most reactive ROS and react at a high rate with almost every biomolecule (Buxton et al., 1988; Karam et al., 1991). Oxidative damage of cell membrane lipids, proteins and nucleic acids are characteristic predictors of hydroxyl radical mediated toxicity and tissue damage (Halliwell, 1992).

The direct measurement of highly reactive radicals, in particular hydroxyl radicals, in vivo is extremely difficult. An elegant method is the salicylate hydroxylation assay (Floyd et al., 1984; Grootveld and Halliwell, 1986). Using this assay in combination with reverse microdialysis (Teismann et al., 2001) or after systemic salicylate injection a single administration of MPTP (30 mg/kg) produced an increase of the indirect hydroxyl radical marker 2,3-dihydroxybenzoic acid (Fig. 3).

Transgenic mice which overexpress the superoxide detoxifying enzyme superoxide dismutase (SOD) showed a significant protection against MPTP toxicity (Przedborski et al., 1992). Also, nitric oxide (NO) seems to be directly and indirectly involved in MPTP neurotoxicity (Castagnoli et al., 1997; Di Monte et al., 1997). Moreover, the reaction of NO with superoxide results in the formation of peroxynitrite (Beckman et al., 1990; Huie and Padmaja, 1993), which is neurotoxic by oxidation and nitration of biomolecules (Beckman, 1996). Peroxynitrite inhibits complex I, II and III of the mitochondrial respiratory chain and irreversibly enhances energy depletion (Radi et al., 1991).

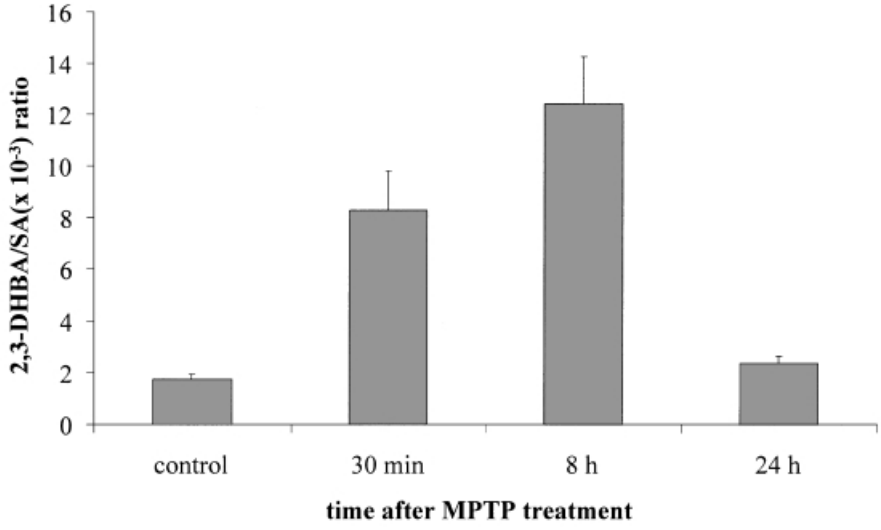

Fig. 3. MPTP-induced hydroxyl radical formation in mice using the salicylate hydroxylation assay. Thirty min before striatal tissue was analyzed for 2,3-dihydroxybenzoic acid (2,3-DHBA) or salicylic acid (SA) mice were injected with SA (100 mg/kg i.p.). MPTP (30 mg/kg s.c.) produced a rapid increase in hydroxyl radical formation which peaked at 8 h after MPTP administration. Mean values ±SEM of n = 4–5 mice are presented (for further information please see Ferger et al., 2000)

In this respect studies using NO synthase inhibitors as well as mice with a deficiency in different isoforms of NO synthetizing enzymes such as nNOS or iNOS showed pronounced protection against MPTP toxicity (Schulz et al., 1995; Hantraye et al., 1996; Przedborski et al., 1996; Liberatore et al., 1999). Recently, 3-nitrotyrosine a marker for nitration reactions was found to be elevated after MPTP administration using the reliable mass spectrometry determination which supports the involvement of reactive nitrogen species (RNS) such as peroxynitrite in MPTP toxicity (Pennathur et al., 1999).

Oxidative stress may also account for the defect of complex I activity by itself, as complex I is highly vulnerable to oxidative damage (Allen et al., 1995). ROS destroy the integrity of the mitochondrial membrane, thus disturbing calcium homeostasis. On the other hand, inhibition of complex I (Cleeter et al., 1992) and increased calcium levels enhance the formation of ROS showing the close interactions which finally result in a self-amplifying vicious circle and cell death.

However, up to date it is impossible to distinguish between a causative role or a consequence of reactive oxygen and nitrogen species involvement in the mechanism of MPTP toxicity. Highly sensitive electron spin resonance spectroscopy (ESR) techniques which do not require the use of invasive application of spin traps but are able to directly measure radicals with high temporal and spatial resolution will improve the understanding of radical involvement and the diagnosis of PD in future. Meanwhile, the detoxification of ROS or RNS at an initial, intermediate or final stage in MPTP toxicity and PD will be a good strategy for protection of dopaminergic neurons against radical attacks.

Cytokines and inflammatory processes

Furthermore, ROS and NO production is enhanced by microglia infiltration and microglia activation as well as inflammatory reactions were determined in the MPTP mouse model (Czlonkowska et al., 1996; Kurkowska-Jastrzebska et al., 1999). Reactive microglia produce secondary cell destruction by releasing cytotoxic species like hydroxyl radicals, NO, glutamate and cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α) (Grünblatt et al., 2000). Indeed, in MPTP treated mice proinflammatory cytokines were elevated (Mogi et al., 1998; Kaku et al., 1999). In a preliminary study we found a significantly higher survival rate and an attenuation of impairments in motor behavior in TNF-α (−/−) mice in comparison to wildtype controls after 4 applications of 20 mg/kg MPTP (2 h apart) (Leng and Ferger, unpublished observations).

In addition, based on post mortem analysis elevated levels of proinflammatory cytokines were demonstrated in parkinsonian brains (Mogi et al., 1994a,b). Furthermore, in persons who inadvertently ingested MPTP up to 14 years ago evidence of chronic inflammation was reported (Langston et al., 1999). In the light of this observation it is not surprising that antiinflammatory drugs such as meloxicam and acetylsalicylic acid showed a pronounced neuroprotection against MPTP toxicity (Teismann and Ferger, 2001).

Salicylic acid which in contrast to acetylsalicylic acid is a weak inhibitor of the enzyme cyclooxygenase, shows pronounced radical scavenging properties in vitro and in vivo. Probably, the protective effect of salicylate against MPTP toxicity in mice (Ferger et al., 1999; Mohanakumar et al., 2000) is related to its capability to detoxify hydroxyl radicals by aromatic hydroxylation reactions which result in di- and trihydroxylated salicylate derivatives. However, higher concentrations of salicylate inhibit translocation of the transcription factor nuclear factor-κB (NF-κB) from the cytoplasm to the nucleus. Salicylate-induced inhibition of NF-κB activation protected against glutamate toxicity (Grilli et al., 1996) and was protective in a mouse model of cerebral ischemia (Schneider et al., 1999). However, after acute administration of MPTP no NF-κB activation was found and mice deficient of the p50 subunit of NF-κB were not protected against MPTP toxicity (Teismann et al., 2001).

Neurochemistry and immunohistochemistry

After uptake of MPP^+ into dopaminergic neurons axonal projections lead to selective damage in the SNc. The degeneration starts a few hours after treatment and lasts up to 4 days. Loss of tyrosine hydroxylase immunoreactivity (TH-IR) not always correlates with reduced numbers of Nissl-stained cells during the first week after MPTP injection (Jackson-Lewis et al., 1995) because the so called ghost cells transiently show no TH-IR but partly recover during the next days. After one week we found a significant and reliable reduction of both, TH-IR and Nissl-stained cells (please see Fig. 4). Additionally, a decreased density of dopamine receptors was found after MPTP treatment (Mitsumoto et al., 1998).

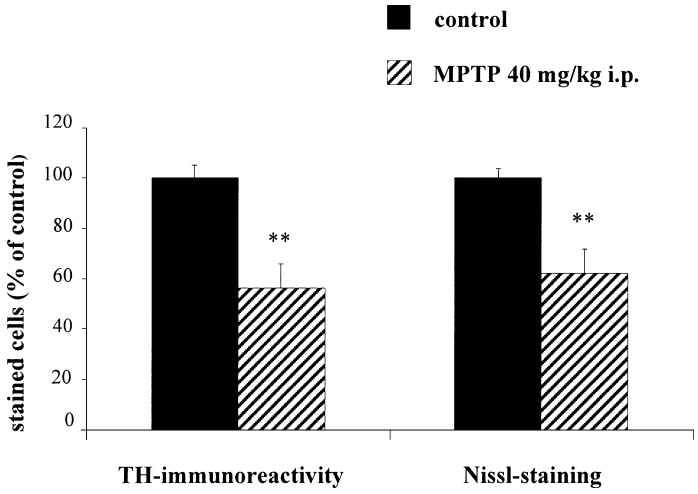

Fig. 4. Effects of MPTP treatment (40 mg/kg i.p.) on TH-immunoreactivity (TH-IR) and Nissl-staining in midbrain sections of mice. MPTP administration led to a significant decrease in the mean number of TH-immunoreactive and Nissl-stained neurons in the substantia nigra pars compacta compared with control values which were set at 100 % (86 ± 4 TH-IR positive cells and 96 ± 4 cells for Nissl-staining per section, respectively). Mean values ±SEM of n = 7–10 mice are presented. ANOVA with subsequent Bonferroni test for comparison of the MPTP treated group compared to controls (**$P < 0.01$) (Schmidt and Ferger, unpublished data, for a detailed description of TH-immunohistochemistry and Nissl-staining please see Schmidt and Ferger, 2001)

MPP^+ acutely displaces dopamine from synaptosomal vesicles and gives raise for dopamine-induced hydroxyl radical formation (Chiueh et al., 1992). Using the reverse microdialysis technique we found a 60-fold increase of dopamine release after striatal application of MPP^+ and significantly increased hydroxyl radical formation (Kuschinsky and Ferger, 1996).

The levels of DA and its metabolites DOPAC and HVA are presented in Fig. 5 seven days after MPTP treatment. MPTP led to the highest reduction in striatal dopamine, followed by the reduction of DOPAC and HVA levels, whereas the serotonergic system was not affected (Fig. 5) which points out the selectivity of this model. In one study we tested the persistence of striatal dopamine depletion 28 days after MPTP treatment and found that there was a slight recovery over time (Fig. 6). The results are in agreement with Mihatsch et al. (1988) who used intracerebroventricular injections of MPP^+ in mice and found a reduction of striatal dopamine levels to 45% at day 4 which partly recovered to 57% 41 days after MPP^+ administration (Mihatsch et al., 1988).

Behavior

Mice show only transient behavioral symptoms as an initial short-term toxic effect of MPTP. They exhibit hypersalivation, piloerection, seizures and hypokinesia and recover within 24–48 h. Moderate dopamine depletion did not

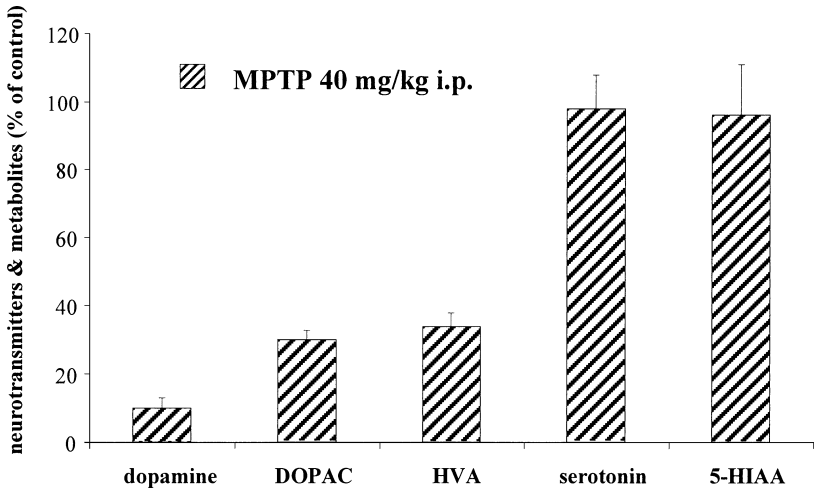

Fig. 5. Effect of MPTP treatment on monoamine and metabolite levels in mice one week after MPTP administration. The following values of the control group which received saline instead of MPTP (40 mg/kg i.p.) were set at 100% for better comparison of the decrease of striatal dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin and 5-hydroxyindoleacetic acid (5-HIAA) levels: DA 15.22 ± 1.96; DOPAC 0.77 ± 0.11; HVA 1.37 ± 0.25; serotonin 0.42 ± 0.12, 5-HIAA 0.31 ± 0.02 ng/mg. Mean values ±SEM of n = 7–10 mice are presented. ANOVA with subsequent Bonferroni test for comparison of MPTP treated animals with controls (***$P < 0.001$) (Schmidt and Ferger, unpublished data, for a detailed description of tissue preparation and HPLC analysis please see Schmidt and Ferger, 2001)

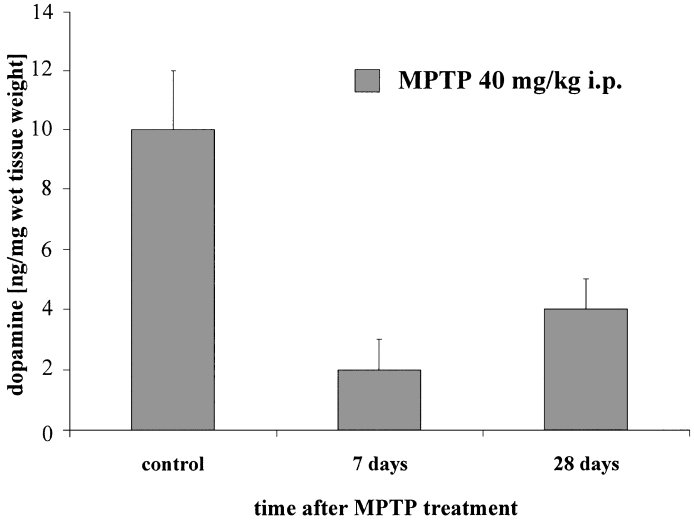

Fig. 6. Comparison of striatal dopamine levels 7 and 28 days after MPTP treatment (40 mg/kg i.p.) in mice. The MPTP-induced dopamine depletion was partly reversible after 28 days. Mean values ±SD of n = 3–4 mice are presented) (Schmidt and Ferger, unpublished data, for a detailed description of tissue preparation and HPLC analysis please see Schmidt and Ferger, 2001)

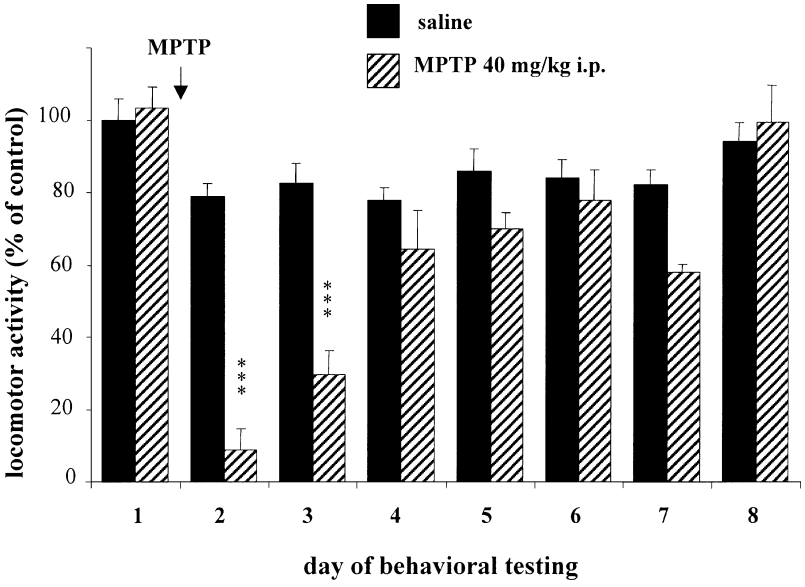

Fig. 7. Effects of MPTP treatment on locomotor activity. MPTP (40 mg/kg i.p.) was injected at day 2 and was able to decrease the locomotor activity on the day of injection to 9.3% and on the following day to 30.5% of control levels. Remarkably, from day 4 up to the end of the behavioral testing the MPTP-induced impairment of locomotor activity was no longer present. Mean values ±SEM of n = 7–10 mice are presented. ANOVA with subsequent Bonferroni test for comparison of the MPTP treated group with controls (***$P < 0.001$) (Schmidt and Ferger, unpublished data, for a detailed description of analysis of locomotor activity please see Teismann and Ferger, 2001)

produce overt motor symptoms, but performance in spatial working memory tasks was impaired when more difficult tasks were applied (Tanila et al., 1998).

Long-term effects including postural abnormalities, bradykinesia, and tremor were found by some groups as a result of dopamine denervation but were not observed by ourselves. As stated above the scheme of MPTP administration and the strain of animals used may lead to apparently different observations. In our hands the decrease of locomotor activity nearly completely recovered 3–7 days after MPTP treatment (Fig. 7).

Conclusions and outlook

Most of the animal models, including the presented MPTP model of PD, are not able to perfectly replicate all features as a counterpart of the human disorder. Indeed, PD is a disorder which mainly affects the elderly and shows a progression over decades, which cannot be achieved in an animal model designed to develop therapeutic strategies. In this review we tried to point out the advantages and limitations using MPTP as a dopaminergic neurotoxin in PD research.

The MPTP model is the most thoroughly investigated model of PD, which does not mean that there is no need to improve its application in future. For

example rotenone, another inhibitor of the complex I of the mitochondrial respiratory chain, showed selective dopaminergic degeneration in the substantia nigra of rats and Lewy body like inclusions (Betarbet et al., 2000). The latter has not been observed in the MPTP mouse model so far. However, rotenone did not work properly in mice (Thiffault et al., 2000). Moreover, a huge variability of rats chronically infused with rotenone indicated by only 50% responders (Betarbet et al., 2000) needs to be elucidated before this model will gain more impact to study neuroprotective drug actions. A breakthrough to mimick the progressive nature of dopaminergic neurodegeneration in PD was the striatal application of 6-OHDA which retrogradely damages the nigrostriatal pathway over weeks (Sauer and Oertel, 1994). Currently, some groups are trying to control the MPTP induced time course of neurodegeneration by using numerous MPTP injections over a time period of more than 3 weeks using low and intermediate MPTP doses in presence and absence of MPTP toxicity enhancers such as diethyldithiocarbamate (DDC), acetaldehyde, probenecid etc. This may alter the acute mode of MPTP toxicity generally observed in the MPTP mouse model during the first days to a more prolonged and progressive neurodegeneration. Less attention was paid in the past to reduce the toxic effects of MPTP in non target organs of the periphery which certainly account for the high mortality in some studies. A better neurochemical monitoring of the pharmacokinetics, in particular uptake, metabolism, and elimination of MPTP/MPP^+ will diminish the huge variations in mortality between different series and different laboratories as well as predict more reliable "real" neuroprotective effects rather than alterations in MPP^+ availability. Parallel cell culture experiments are not sufficient to study the pharmacokinetics of MPTP toxicity. Instead, for example HPLC measurements of striatal MPP^+ concentrations or uptake studies with radioactive labeled MPP^+ in vivo have to be performed.

Nevertheless, the MPTP model provided important contributions towards a better understanding of the mechanisms involved in nigrostriatal degeneration in PD because it is highly practicable and adequately mimicks the neurochemical, neuroanatomical and some of the behavioral characteristics of PD. However, as recently reviewed by Przedborski and coworkers (2001), the experimenter should always be very careful and strictly follow the safety rules using MPTP as it is one of the most harmful compounds in experimental research. Otherwise one risks to become a victim after years of practice.

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