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Neuroprotection by dopamine agonists

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Summary. Research on Parkinson's disease has led to new hypotheses concerning the mechanisms of neurodegeneration and to the development of neuroprotective agents. Recent findings of impaired mitochondrial function, altered iron metabolism and increased lipid peroxidation in the substantia nigra of parkinsonian patients emphasize the significance of oxidative stress and free radical formation in the pathogenesis of Parkinson's disease. Present research is therefore focussing on improvements in neuroprotective therapy to prevent or slow the rate of progression of the disease. Possible neuroprotective strategies include free radical scavengers, monoamine oxidase-B inhibitors, iron chelators and glutamate antagonists.

Recent studies point to the possibility of achieving neuroprotection in ageing and parkinsonism by the administration of dopamine agonists. In the rat, the dopamine agonist pergolide appears to preserve the integrity of nigrostriatal neurones with ageing. The prevention of age-related degeneration may be achieved as a result of a decreased dopamine turnover and reduced conversion of dopamine to toxic compounds. In our own study, bromocriptine treatment prevented the striatal dopamine reduction following MPTP administration in the mouse. These results suggest that the neurotoxic effects of MPTP can be prevented by bromocriptine. Monotherapy with the dopamine agonist lisuride in the early stages of Parkinson's disease delays the need for the initiation of levodopa treatment to a similar extent as has been reported for L-deprenyl. It remains to be shown whether this is due to neuroprotective efficacy of the dopamine agonist or to a direct symptomatic effect.

Introduction

The most important neuropathological feature of Parkinson's disease is the loss of catecholaminergic neurones in the brainstem (German et al., 1989;

Jellinger, 1991). The degeneration of the melanin-pigmented dopaminergic neurones of the substantia nigra pars compacta is the pathological basis of the movement disorders characterizing Parkinson's disease. The cause of degeneration of dopamine-containing neurones in Parkinson's disease remains unknown. The finding that dopamine levels are reduced in the striatum and substantia nigra of parkinsonian subjects (Ehringer and Hornykiewicz, 1960) has led to the introduction of replacement therapies including L-DOPA (Birkmayer and Hornykiewicz, 1961), dopamine full (Calne et al., 1974; LeWitt, 1986; Sage and Duvoisin, 1985) and partial agonists (Lange et al., 1992b), and of the monoamine oxidase type B (MAO-B) inhibitor L-deprenyl (Birkmayer et al., 1975).

The selective vulnerability of the nigrostriatal dopamine-containing neurones to the toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and the striking resemblance of the resulting clinical syndrome to Parkinson's disease has focussed research activity on the search for aetiological factors that may contribute to the development of Parkinson's disease.

Pathogenesis of Parkinson's disease

The discovery that the neurotoxin MPTP induces neuropathological and neurochemical alterations as well as clinical signs very similar to those of Parkinson's disease (Ballard et al., 1985; Burns et al., 1985) suggests that there are neurotoxic compounds in the environment similar to MPTP. Although there is some epidemiological evidence in support of a role of environmental neurotoxins (Snyder and D'Amato, 1986; Tanner and Langston, 1990; Riederer and Lange, 1992), no such toxic agent has been identified. Even if MPTP and related substances are not the cause of Parkinson's disease, MPTP neurotoxicity provides clues as to the mechanism underlying neuronal death in this disease.

The discovery of a defect in mitochondrial electron transport at complex I in the substantia nigra of parkinsonian patients (Schapira et al., 1990) has led to the hypothesis that a mitochondrial abnormality can increase the vulnerability of some individuals to neurodegeneration involving the substantia nigra. The complex I deficiency may be caused by an MPTP-like substance or may be determined genetically. Putative mitochondrial abnormalities may not be the primary aetiological factor but could be secondary to another metabolic deficit, e.g. excess free radicals produced from other sources than complex I inhibition may cause the complex I deficiency reported in Parkinson's disease.

It has been suggested that oxidative stress or excess free radical formation play a role in the pathogenesis of Parkinson's disease (Graham et al., 1978; Spina and Cohen, 1989; Youdim et al., 1990). There are two theoretical concepts of how oxidation reactions and toxic oxygen species may contribute to the degenerative process underlying neuronal death in Parkinson's disease.

The first concept proposes that excess formation of free radicals occurs as a result of toxin action. The neurotoxin MPTP expresses its toxicity as a consequence of its oxidation to the 1-methyl-4-phenyl-pyridinium ion (MPP^+) by monoamine oxidase type B (MAO-B) (Chiba et al., 1984; Salach et al., 1984; Heikkila et al., 1985). Inhibition of the oxidation of MPTP to MPP^+ by MAO-B inhibitors such as deprenyl and pargyline prevents the neurotoxic effects of MPTP and the development of a parkinsonian syndrome in animal models of Parkinson's disease (Cohen et al., 1984; Heikkila et al., 1984; Langston et al., 1984b). If Parkinson's disease is caused by MPTP-like compounds, oxidation reactions may be essential for the development of Parkinson's disease. The toxic effects of MPP^+ are thought to be caused by its ability to inhibit complex I of the mitochondrial respiratory chain (Nicklas et al., 1985; Vyas et al., 1986) resulting in decreased cellular adenosine triphosphate levels (DiMonte et al., 1986) and altered intracellular calcium content (Kass et al., 1988). Alterations in the homoeostasis of intracellular calcium are closely linked with altered cell function and cell death (Orrenius et al., 1989). Recent studies have shown that the calcium binding protein calbindin is selectively decreased in the substantia nigra in Parkinson's disease (Iacopino and Christakos, 1990). The inhibition of mitochondrial function may also lead to increased formation of free radical species.

The second concept concerning the importance of oxidation reactions and free radical formation in the pathogenesis of Parkinson's disease relates to the metabolism of dopamine. Several lines of evidence suggest that dopamine or products of dopamine metabolism are neurotoxic. It has been shown, for example, that dopamine depletion has a protective effect on ischaemia-induced striatal damage. Brain anoxia causes a release of large quantities of dopamine in the gerbil (Brannan et al., 1987). In the rat, the unilateral destruction of the nigrostriatal dopaminergic pathway by 6-hydroxydopamine protects striatal neurones on the ipsilateral side against damage from global forebrain ischaemia (Globus et al., 1987a,b; Clemens and Phebus, 1988). The hypothesis of a neurotoxic role of dopamine is supported by the finding that dopamine depletion by alpha-methylparatyrosine protects the dopamine re-uptake mechanism of striatal nerve endings against destruction by ischaemia (Weinberger et al., 1985). These results suggest that, at least in cerebral ischaemia, dopamine is involved in the process leading to neuronal cell death.

Several neurochemical characteristics of the substantia nigra may enhance free radical formation and contribute to oxidative stress vulnerability. Dopamine can be oxidatively metabolized by the enzyme monoamine oxidase (MAO). The polymerization of auto-oxidative products of dopamine leads to the formation of neuromelanin and the characteristic pigmentation of the substantia nigra. Both auto-oxidation of dopamine and oxidative deamination by MAO result in the formation of hydrogen peroxide (H_2O_2). Under normal circumstances H_2O_2 is rather inert and never accumulates in the brain or other organs. H_2O_2 is normally cleared from the brain by the glutathione system. Glutathione peroxidase catalyzes

the reaction of H_2O_2 with glutathione (GSH) to form glutathione disulfide (GSSG):

$$H_2O_2 + 2GSH \xrightarrow[\text{peroxidase}]{\text{Glutathione}} 2H_2O + GSSG$$

In the presence of iron, H_2O_2 can be reduced to form the toxic hydroxyl free radical (Fenton reaction):

$$H_2O_2 + Fe^{2+} \longrightarrow \cdot OH + OH^- + Fe^{3+}$$

MAO activity in the brain increases with ageing (Fowler et al., 1980) and this may lead to an increase in the formation of H_2O_2 which could exceed the capacity of the glutathione system. Similarly, a reduction in glutathione or glutathione peroxidase could prevent the clearance of H_2O_2 generated from normal dopamine metabolism (Sofic et al., 1992). The result of either of these mechanisms could be the insufficient clearance of H_2O_2 and the production of hydroxyl free radicals which may cause damage to dopamine-containing cells.

Within the brain, high concentrations of iron have been shown to exist in the substantia nigra and striatum (Hill and Switzer, 1984; Riederer et al., 1989). Free tissue metals such as Fe^{2+} can initiate the formation of cytotoxic oxygen free radicals resulting from their interaction with hydrogen peroxide. This leads to promotion of membrane lipid peroxides. A selective increase in iron content occurs in the substantia nigra in Parkinson's disease (Riederer et al., 1989; Dexter et al., 1989; Jellinger et al., 1990; Sofic et al., 1991). Melanin-iron interaction leads to a potentiation of iron-induced basal lipid peroxidation (Ben-Shachar and Youdim, 1990). These findings suggest that oxidative stress induced by iron-melanin interaction is a possible mechanism in the aetiology of Parkinson's disease without involving an endogenously or exogenously derived neurotoxin (Youdim et al., 1989).

Table 1. Evidence supporting a state of oxidative stress in the substantia nigra in Parkinson's disease

Disturbed mitochondrial respiratory function with reduction in complex I and III activities
Altered cellular calcium homoeostasis with decrease in calcium-binding protein
Decreased glutathione and glutathione peroxidase activity leading to a reduced ability to scavenge hydrogen peroxide derived from oxidative deamination and auto-oxidation of dopamine
Increased iron content resulting in a potential excess of radical-generating free iron
Increased mitochondrial superoxide dismutase activity, perhaps reflecting an attempt to compensate for oxidative stress
Increased peroxidation of membrane lipids inducing membrane damage and cell death

Recent research on the biochemical pathology of Parkinson's disease indicates that free radicals generated from oxidation reactions play an important role in the neuronal loss in the substantia nigra in Parkinson's disease (for review see Lange et al., 1992a, and Table 1).

Neuroprotective strategies in Parkinson's disease

The biochemical alterations in Parkinson's disease such as increased lipid peroxidation, altered iron metabolism and impairment of mitochondrial function point to oxidative stress as an important factor contributing to neuronal loss in the substantia nigra in Parkinson's disease. Protection against such oxidative damage could be provided by scavengers of free radicals and anti-oxidants such as MAO-B inhibitors, alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), glutathione and iron chelators (see Table 2).

Treatment with ascorbic acid in the mouse has been reported to reverse the decrease in striatal dopamine levels caused by the systemic administration of MPTP or focal injection of MPP^+ into the striatum (Sershen et al., 1985; Wagner et al., 1985, 1986). Alpha-tocopherol has been shown to prevent nigral cell loss in MPTP-treated mice (Perry et al., 1985) and has been reported in an open-label study to retard the clinical progression of Parkinson's disease in therapeutically naive patients (Fahn, 1989). Another open study showed that parkinsonian patients taking alpha-tocopherol had less severe deficits than those not taking the substance (Factor et al., 1990). The DATATOP study has compared the effect of alpha-tocopherol and placebo on disease progression in a prospective, double-blind trial and has found that tocopherol produced no beneficial effects (Parkinson Study Group, 1989, 1993).

The findings of a selective increase in oxidative stress and in Fe^{3+} content in the substantia nigra in Parkinson's disease suggest that iron chelators may be able to prevent the retardation of the dopaminergic neurodegeneration. The dopaminergic neurotoxic effect of 6-hydroxydopamine is thought to involve the generation of oxygen free radicals (Heikkila and Cohen, 1972; Sachs and Jonsson, 1975; Graham et al., 1978) presumably initiated by a transitional metal. The administration of the iron chelator desferrioxamine in the rat protects against the 6-hydroxydopamine-induced reduction in striatal dopamine content and the development of dopamine-related behavioural changes (Ben-Shachar et al., 1991). The ability of iron chelators to retard dopaminergic neurodegeneration in the substantia nigra may point to a new neuroprotective approach in Parkinson's disease. Iron chelators such as amino steroids have shown protective activity in animal models of trauma and ischaemia and are able to cross the blood-brain barrier and to inhibit iron-dependent lipid peroxidation (Braughler et al., 1987; Hall, 1988; Hall and Yonkers, 1988).

The selective MAO-B inhibitor L-deprenyl was initially employed as an adjunct to L-DOPA, based on the hypothesis that inhibition of dopamine

metabolism would increase dopamine availability. MAO-B inhibitors increase brain levels of dopamine and phenylethylamine (Neff et al., 1974; Riederer et al., 1984) and L-deprenyl potentiates the anti-parkinsonian action of L-DOPA (Birkmayer et al., 1975; Elizan et al., 1991). Retrospective studies showed that patients who received both L-DOPA and L-deprenyl lived longer than patients who were treated with L-DOPA alone (Birkmayer et al., 1985). Two randomized, prospective, double-blind studies have compared L-deprenyl with placebo in otherwise untreated subjects with early Parkinson's disease (Parkinson Study Group, 1989, 1993; Tetrud and Langston, 1989). Both studies demonstrated that L-deprenyl produced a prolongation of the period before systematic therapy was required. L-deprenyl appears to delay the onset of disability and to have a neuroprotective effect by slowing the rate of progression of Parkinson's disease in newly diagnosed patients.

L-deprenyl may decrease the generation of hydrogen peroxide associated with dopamine catabolism through its action as an MAO-B inhibitor and slow the progression of Parkinson's disease by reducing the death of substantia nigra neurones induced by endogenous neurotoxic free radicals. A neuroprotective role of L-deprenyl has been demonstrated in the mouse. L-Deprenyl reduced oxidative stress associated with an increased turnover of dopamine induced by haloperidol and limited the accumulation of GSSG in the striatum (Cohen and Spina, 1989).

The hypothesis of neuroprotective efficacy of L-deprenyl has been questioned recently (Landau, 1990; Ward, 1994), since the reduced probability of reaching the endpoint, i.e. the decision to treat with levodopa, may have been due to a direct treatment effect rather than to neuroprotection.

The inhibition of MPTP-induced neurotoxicity by L-deprenyl given prior to the toxin is well established. It has recently been shown that L-deprenyl increases the survival of substantia nigra neurones in the mouse even when the drug is administered days following the MPTP treatment (Tatton and Greenwood, 1991). This finding suggests a neuroprotective mechanism that is independent of MAO-B activity. It can rather be related to the stimulation of neurotrophic factors or regenerative processes than to MAO-B activity.

Neuroprotective activity may be a generalized feature of both MAO-A and MAO-B inhibitors. The selective reversible MAO-A inhibitor moclobemide (p-chloro-N-[2-morpholinoethyl]benzamide) has been reported to have neuroprotective effects due to the inhibition of generation of hydrogen peroxide via MAO-A reactions (Da Prada et al., 1990).

There is evidence indicating that excitatory amino acids are involved in the neurotoxic effects of MPTP. Systemic administration of MPTP to humans and non-human primates causes parkinsonian motor deficits associated with a selective destruction of dopamine-containing neurones in the substantia nigra pars compacta and a marked reduction in striatal dopamine content (Davis et al., 1979; Langston et al., 1983, 1984a; Burns et al., 1983). Neurotoxicity appears to be due to the formation of 1-methyl-4-phenylpyridinium ion (MPP^+) (Castagnoli et al., 1985; Sanchez-Ramos et

Table 2. Neuroprotective strategies in Parkinson's disease

Biochemical alterations in the substantia nigra	Possible neuroprotective therapies
Formation of hydrogen peroxide	MAO-B inhibitors
Increased iron content	Iron chelators
Formation of toxic oxygen free radicals	Free radical scavengers
Alteration in the homoeostasis of intracellular calcium	Calcium entry blockers
Increased dopamine turnover	Dopamine agonists
Excess activity of excitatory amino acids (?)	Excitatory amino acid antagonists

al., 1988; Lange, 1990) which is the result of the conversion of MPTP by MAO-B into the dihydropyridinium species ($MPDP^+$) which is converted non-enzymatically into MPP^+. This compound is subsequently transported by the dopamine uptake process to accumulate within dopaminergic neurones and to be temporarily stored in a releasable pool (Javitch et al., 1985; Schinelli et al., 1988). The toxicity of MPP^+ apparently occurs as the result of intraneuronal uptake by a mitochondrial carrier and inhibition of complex I of the mitochondrial respiratory chain (Nicklas et al., 1985).

Excitatory amino acids such as glutamate appear to be involved in the pathophysiological cascade of MPTP/MPP^+-induced neuronal death. It has been shown that MPP^+ causes a release of glutamate and aspartate in the rat brain (Carboni et al., 1990). Gutamate antagonists, which competitively or non-competitively block the NMDA subtype of receptor, protect dopaminergic nigral neurones against destruction by MPP^+ injected directly into the substantia nigra pars compacta (SNC) of rats (Turski et al., 1991). Since rats are less sensitive to MPP^+ than primates, the doses of the toxin needed to produce brain damage are very high and could cause unspecific toxic effects (Harik et al., 1987). In the mouse, the non-competitive NMDA-receptor antagonist (+)-5-methyl-10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801) has been shown to be ineffective in preventing the dopamine depletion induced by systemic administration of MPTP (Sonsalla et al., 1989, 1992). Recent studies in monkeys, however, have demonstrated that glutamate antagonists are able to modulate the neurotoxicity of MPTP. The competitive NMDA antagonist 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) protects tyrosine hydroxylase (TH)-positive neurones in the substantia nigra from degeneration induced by systemic treatment with MPTP in the common marmoset (Lange et al., 1993). The non-competitive NMDA antagonist MK-801 prevents the development of the parkinsonian syndrome in the cynomolgus monkey (Zuddas et al., 1992) and protects nigral tyrosine hydroxylase-positive neurones in cynomolgus monkeys (Zuddas et al., 1992) and marmosets (Lange et al., unpublished observation) from degeneration following the administration of MPTP.

The neuroprotective action of competitive and non-competitive NMDA receptor antagonists against MPTP toxicity supports the hypothesis that NMDA receptor-mediated events are involved in the neurotoxicity of MPTP and MPP^+. MPP^+ interferes with mitochondrial respiration and depletes cell energy resources (Nicklas et al., 1985). Neuronal energy deprivation could alter the normal functioning of cell membranes and cause a partial depolarization leading to a release of the voltage dependent Mg^{2+} block of NMDA receptor ion channels (Nowak et al., 1984). Removal of the Mg^{2+} block enables excitatory amino acids to excite their receptors persistently, to open the ion channels and to become neurotoxic (Novelli et al., 1988).

Pergolide and ageing

As has been described above, dopamine can play a neurotoxic role in ischaemic brain damage. Recent studies have addressed the question of whether dopamine may be involved in the age-related decline of the dopaminergic nigrostriatal system and the destruction of dopamine-containing neurones. This hypothesis can be tested by a chronic reduction of dopamine synthesis and release over the entire life-span of an animal. This can be achieved by a continuous stimulation of the dopamine auto-receptors causing a decrease in dopamine availability. Pergolide is not only a potent dopamine agonist at postsynaptic dopamine receptors but also a potent agonist at presynaptic dopamine autoreceptors (Fuller et al., 1982). This compound is not subject to auto-oxidation and the production of toxic reactive oxygen intermediates.

The effects of dietary administration of pergolide for two years on alterations of the nigrostriatal system have been observed in the rat (Felten et al., 1992). Fischer-344 rats were fed a diet containing 0.001% of pergolide from three months of age until the age of 26 months. A control group was pair-fed with the pergolide group in order to control for food consumption and body weight. Some animals were killed at 18 months of age, i.e. after 15 months of continuous pergolide administration. In comparison to control animals, rats on pergolide treatment for 15 months showed a reduced dopamine turnover in the striatum as expressed by markedly lower levels of 3,4-dihydroxyphenylacetic acid (DOPAC). At 26 months of age both the pergolide group and the pair-fed control group were killed and compared with a group of young rats aged three months. The density of cell bodies in the substantia nigra pars compacta, determined with fluorescence histochemistry, was reduced in the 26-month-old pair-fed control rats when compared with both 26-month-old pergolide-treated group and the 3-month-old control group. The relative density of cell bodies in the substantia nigra of the rats treated with pergolide did not differ from that of the young control rats aged three months (Felten et al., 1992). The density of dopamine terminals in the rostral striatum of the pergolide-treated group did not differ from the 3-month-old control group. The 26-month-old pair-fed control

group, however, showed a clearly reduced density of striatal dopamine terminals. These results suggest that chronic pergolide administration is able to preserve the integrity of the dopaminergic nigrostriatal system during ageing. There was no difference in tissue content of dopamine and DOPAC when 3-month-old control rats and 26-month-old pergolide-treated and pair-fed animals were compared (Felten et al., 1992). Since the number of dopaminergic neurones was reduced in the pair-fed group, each neuron must have synthesized more dopamine to compensate. In the neurochemical analyses the total tissue content of dopamine was measured whereas in the histochemical fluorescence studies only intracellular dopamine was determined and extracellular dopamine did not contribute to the fluorescence. The dopaminergic neurones in old animals therefore seem to produce more dopamine to compensate for the loss of neurones.

In the rat pergolide appears to preserve the integrity of nigrostriatal neurones with ageing. The prevention of age-related degeneration may be the result of a decreased dopamine turnover and reduced conversion of dopamine to toxic compounds. Pergolide has been shown to induce superoxide dismutase in the rat striatum (Clow et al., 1992). This effect may help to protect against nigrostriatal degeneration.

Bromocriptine and MPTP neurotoxicity

The MPTP animal model offers the opportunity to investigate possible neuroprotective effects of compounds in an experimental parkinsonian syndrome. We have tested whether the dopamine agonist bromocriptine can influence the neurotoxicity of MPTP in the mouse.

Male C57/B16 mice (Charles River, Sulzfeld, Germany) weighing 18–20 g were used. They were housed under standard laboratory conditions (12 h light, 12 h darkness, food pellets and tap water ad libitum).

One group of mice (n = 17) received bromocriptine (100 mg/kg) dissolved in 0.2 ml of 5% gum arabic solution through a pharyngeal tube twice daily for 3 days (treatment schedule see Table 3). Sixty minutes later diethyldithiocarbamate (400 mg/kg, Sigma) dissolved in 0.2 ml saline was injected intraperitoneally. Thirty minutes following the DDC administra-

Table 3. Treatment schedule for C57/B16 mice

Time	7.30	8.30	9.00	19.30	20.30	21.00
Day 1	BROMO	DDC	MPTP	BROMO	DDC	MPTP
Day 2	BROMO	DDC	MPTP	BROMO	DDC	MPTP
Day 3	BROMO	DDC	MPTP	BROMO	DDC	MPTP
Day 7	Decapitation					

BROMO bromocriptine (100 mg/kg); *DDC* diethyldithiocarbamate (400 mg/kg); *MPTP* 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (12 mg/kg)

tion, MPTP hydrochloride (12 mg/kg, Paesel, Austria) dissolved in 0.2 ml saline was injected i.p.

Another group of mice (n = 19) was treated with DDC and MPTP according to the above treatment schedule and received vehicle instead of bromocriptine. A third group (n = 4) was treated with bromocriptine only and injected with vehicle instead of DDC and MPTP. The final control group (n = 30) did not receive any active compound but was injected with vehicle according to the same time schedule as the other three groups.

Six days following the first treatment all animals were decapitated. The brains were immediately removed and the cortex, striatum and substantia nigra were dissected. The brain tissue samples were stored at −80°C until further processing. For neurotransmitter analysis tissue samples (2–20 mg) were homogenized with a sonicator in 0.2 ml of 0.4 M $HClO_4$ for 30 seconds, the homogenates were then centrifuged at 3,000 × g for 5 minutes. The supernatant was dissolved in 1 ml of 1 M Tris buffer (pH 8.66), 800 pg

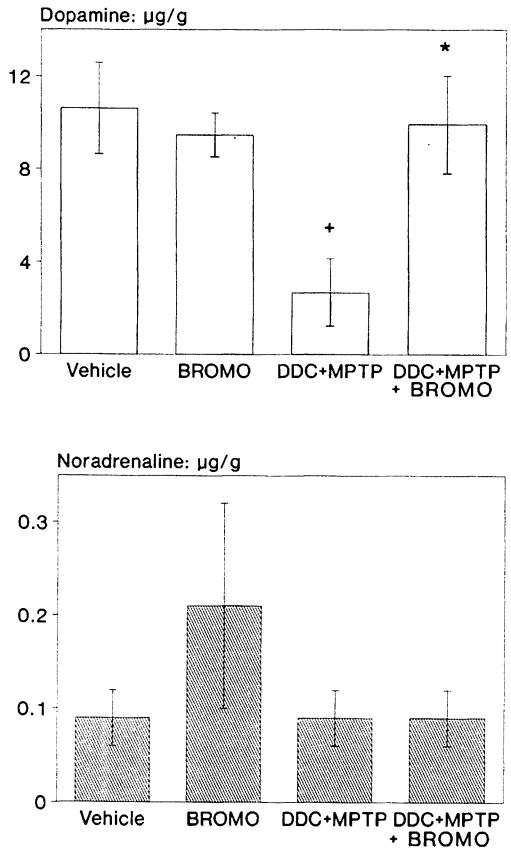

Fig. 1. Dopamine and noradrenaline levels (mean ± S.D.) in the striatum of mice treated with vehicle, bromocriptine (100 mg/kg twice daily for 3 days), DDC (400 mg/kg twice daily for 3 days) plus MPTP (12 mg/kg twice daily for 3 days) or bromocriptine plus DDC plus MPTP. †$p < 0.01$ for comparison with vehicle group, *$p < 0.01$ for comparison with DDC+MPTP group (Welch t-test)

of 3,4-dihydroxybenzylamine-HBr (DHBA) were added as an internal standard. The catecholamines were extracted in 50 mg of acid-washed alumina (Merck) by shaking for 15 minutes. The supernatant was aspirated and the alumina was washed with 1 ml of 0.1 M Tris buffer and then with 1 ml of distilled water. The bound catecholamines were dissolved with 0.2 ml of 0.4 $HClO_4$. Following centrifugation at 12,000 × g, 25 µl were injected into an HPLC system consisting of an HPLC pump (ESA-5700, Bedford, U.S.A.; ESA catecholamine HR-80 column, length 80 mm, 3 µm spherical octadecylsilane) and an electrochemical detector (ESA-5100A; potentials for guard cell 0.10 V, detector 1 0.35 V, detector 2 0.20 V). The solvent used was Cat-A-Phase (ESA). Dopamine and noradrenaline were separated at a flow of 1 ml/min according to the method described by Sofic (1986). Catecholamine levels in the brain regions examined were compared using the Welch t-test.

In comparison with the animals treated with vehicle, the administration of DDC plus MPTP caused a decrease of dopamine in the striatum of about

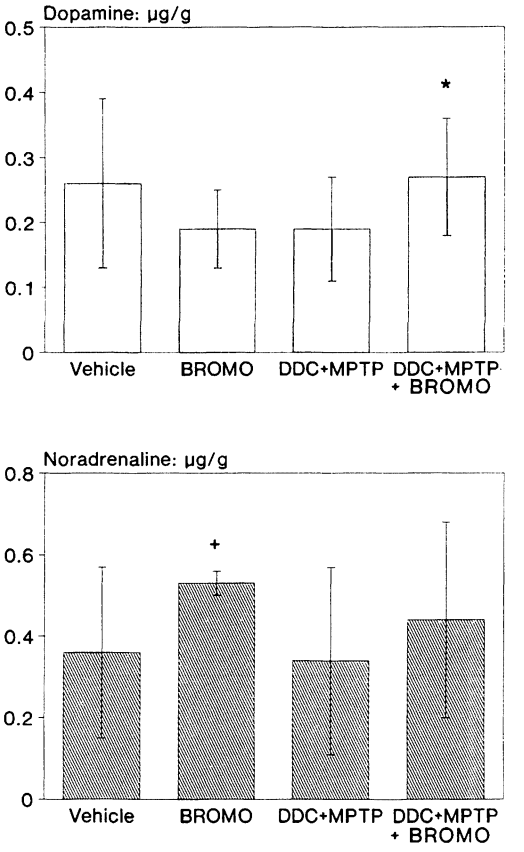

Fig. 2. Dopamine and noradrenaline levels (mean ± S.D.) in the substantia nigra of mice treated with vehicle, bromocriptine (100 mg/kg twice daily for 3 days), DDC (400 mg/kg twice daily for 3 days) plus MPTP (12 mg/kg twice daily for 3 days) or bromocriptine plus DDC plus MPTP. †$p < 0.01$ for comparison with vehicle group, *$p < 0.01$ for comparison with DDC+MPTP group (Welch t-test)

70% (see Fig. 1). Treatment with DDC, MPTP and bromocriptine was associated with striatal dopamine levels that were higher than those in the DDC plus MPTP group and comparable to those in the control groups treated with vehicle or bromocriptine alone (see Fig. 1). Striatal noradrenaline levels did not differ between the experimental and control groups. Dopamine content in the substantia nigra was not significantly reduced following DDC plus MPTP compared to the vehicle group (see Fig. 2). Treatment with DDC plus MPTP plus bromocriptine, however, caused higher dopamine levels than the administration of DDC and MPTP (Fig. 2). Apart from an increase in noradrenaline following bromocriptine in comparison with vehicle administration, there were no differences between the treatment groups. Cortical dopamine and noradrenaline levels were reduced following the treatment with DDC and MPTP in comparison with the vehicle control group (see Fig. 3).

The administration of DDC and MPTP in mice caused a decrease in dopamine content in the striatum and cortex. Additional treatment with

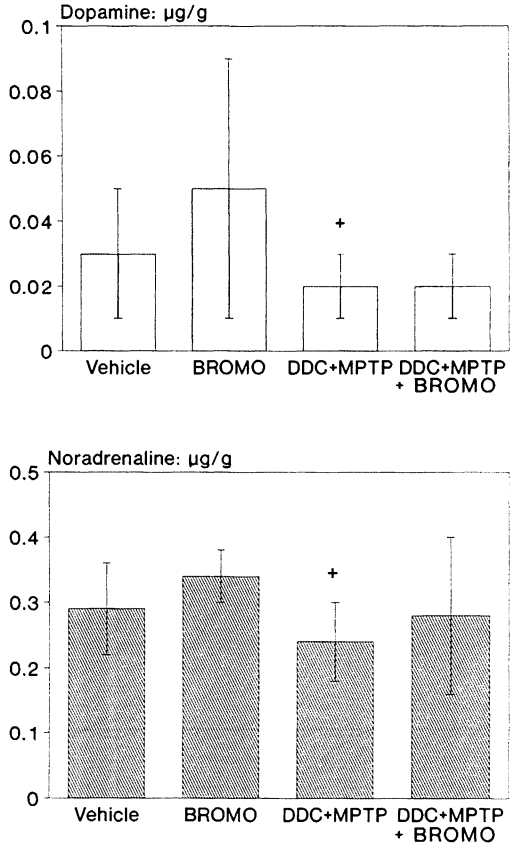

Fig. 3. Dopamine and noradrenaline levels (mean ± S.D.) in the cortex of mice treated with vehicle, bromocriptine (100 mg/kg twice daily for 3 days), DDC (400 mg/kg twice daily for 3 days) plus MPTP (12 mg/kg twice daily for 3 days) or bromocriptine plus DDC plus MPTP. †$p < 0.01$ for comparison with vehicle group (Welch t-test)

bromocriptine prevented the dopamine reduction in the striatum and produced higher dopamine levels in the substantia nigra than following DDC plus MPTP. These results suggest that the neurotoxic effects of MPTP in the mouse can be prevented by bromocriptine. At present it is not clear how dopamine agonists such as bromocriptine protect dopaminergic neurones against the neurotoxic effects of MPTP. The neuroprotective effect of bromocriptine could be brought about by a reduction in dopamine turnover and dopamine uptake and a reduced uptake of MPTP into dopamine-containing neurones. Dopamine agonists may also play a role as free radical scavengers. Further studies should address the question of whether bromocriptine has neuroprotective efficacy in parkinsonian patients.

Dopamine agonists in Parkinson's disease

In a retrospective analysis, the effects of lisuride monotherapy on the need for levodopa therapy has been investigated (Runge and Horowski, 1991). The clinical observations in 185 parkinsonian patients treated with lisuride alone showed a significant lengthening of the period before levodopa therapy was needed. The initiation of levodopa administration could be postponed for a year or longer in about 60% of patients. After several years most patients required levodopa as an additional or alternative treatment. About 10% of the patients showed satisfactory efficacy of lisuride monotherapy for more than five years. The clinical results with lisuride monotherapy (Runge and Horowski, 1991) were comparable to those obtained with L-deprenyl in the DATATOP study (Parkinson Study Group, 1989). Symptomatic therapy with the dopamine agonist lisuride in early Parkinson's disease is able to postpone the need for levodopa therapy to a similar extent as has been reported for the MAO-B inhibitor L-deprenyl. In two preliminary notes, the absence of observable clinical progression of Parkinson's disease has been reported for patients receiving pergolide for up to seven years in addition to levodopa therapy (Lichter et al., 1988; Zimmerman and Sage, 1991).

The results concerning lisuride and pergolide in Parkinson's disease need confirmation by prospective studies. If dopamine agonists slow the progression of Parkinson's disease, this may be caused by the stimulation of the presynaptic autoreceptor and the reduction of the dopamine and free radical load on the nigrostriatal system. This may be true for patients in the early stages without levodopa therapy. However, in parkinsonian subjects taking high doses of levodopa, it would seem impossible that the administration of dopamine agonists could decrease the free radical load to a sufficient extent.

The question that remains to be answered is whether delaying the need for levodopa therapy is a suitable parameter for the evaluation of the progression of the disease and therefore of a possible neuroprotective efficacy of a drug, since there is no evidence showing that lisuride acts on the neurodegenerative process directly. In the case of L-deprenyl, a favour-

able effect on the progression of Parkinson's disease has been postulated (Parkinson Study Group, 1989, 1993).

A major argument against the neuroprotective action of L-deprenyl in the trial of the Parkinson Study Group (1989) has been the short wash-out period of one month. It has been argued that the symptomatic effects of the drug may still have been apparent (Landau, 1990). However, an increase in the concentration of amines is observed only following MAO inhibition of about 80% (Green and Youdim, 1976). Therefore, the symptomatic effect of an MAO inhibitor is lost relatively rapidly as the enzyme recovers from total blockade. New protein synthesis to levels of enzyme protein that sufficiently metabolize the amine takes place in rats within the first two weeks, in monkeys within four weeks and in humans within an unknown period following cessation of an irreversible MAO inhibitor. However, urinary phenylethylamine concentrations, which increase 20 to 90-fold following L-deprenyl administration, drop to normal excretion levels within a few days following L-deprenyl withdrawal (Elsworth et al., 1978). Nevertheless the design of the L-deprenyl study (Parkinson Study Group, 1989, 1993) has been questioned with regard to the assessment of neuroprotective properties of the drug.

With regard to the possible neuroprotection by dopamine agonists in Parkinson's disease, a clear distinction between neuroprotective and symptomatic effects could be made only by the administration of a dopamine agonist without any effects on the parkinsonian symptoms (Lange and Riederer, 1994b).

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