

MPTP-induced degeneration: interference with glutamatergic toxicity

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Summary. Parkinson's disease (PD) is characterised by the progressive degeneration of nigrostriatal dopamine (DA) neurons resulting in the major symptoms of akinesia and rigidity. Although the primary cause of PD is still not known some features make this disorder a model for neurodegenerative diseases in general. It has been known for some time that symptomatic PD can be attributed to insults with symptoms occurring many years later such as post-encephalitic PD or PD following manganese poisoning. More recently, MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been identified as a neurotoxin selective for melanin-containing dopaminergic neurons in humans and non-human primates. The specificity of this neurotoxin and the striking clinical similarities to idiopathic PD, seen in primates, make MPTP-induced parkinsonism the most useful animal model of a neurological disease. There are numerous theoretical possibilities to interfere with both MPTP-induced neurotoxicity and the symptomatology of PD. In recent years excitatory amino acids have gained considerable interest since they can cause excitotoxic lesion of neurons under a number of pathological conditions (Olney et al., 1989; Choi, 1988). Here we summarise the present data and provide new experimental evidence indicating that MPTP-induced degeneration of dopaminergic neurons does involve glutamate-mediated toxicity. It is concluded that glutamate-mediated excitotoxicity results in the destruction of DAergic somata in the substantia nigra. Non-competitive or competitive NMDA antagonists protect nigral neurons from MPTP-induced degeneration whereas their striatal terminals still seem to degenerate.

Excitatory amino acids (EAA) such as L-glutamate or L-aspartate have been identified as transmitters in the mammalian central nervous system (Fonnum, 1984; Watkins et al., 1990). Biochemical, electrophysiological and neuroanatomical studies indicate that both amino acids, beside their metabolic functions, could serve as neuro transmitters. EAAs are released from slices or synaptosome preparations in a calcium-dependent fashion and are accumulated by a high-affinity uptake system. Upon release EAAs act

as depolarizing agents by activating ionophore-coupled glutamate receptors divided pharmacologically into three subtypes. N-methyl-D-aspartate (NMDA), quisqualate (QUIS) or α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and kainate (KAIN) act as agonists on these receptors. Additionally, two G protein-coupled EAA receptors have been identified. The metabotropic receptor, linked to phospholipase C, is activated by trans-1-aminocyclopentane-1,3-dicarboxylate (ACPD). Stimulation of this subtype causes an increased inositol-1,4,5-triphosphate (IP₃) synthesis and a subsequent rise in intracellular calcium levels. Finally, there is a less well-defined receptor, activated by L-2-amino-4-phosphobutyrate (L-AP4), which may be located presynaptically and inhibits transmitter release, again via a presumably G protein-mediated process. Molecular biology revealed the structure of EAA receptors, encoded by a variety of separate genes.

Neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases or amyotrophic lateral sclerosis have been related to dysfunction of glutamatergic systems (Olney, 1989). L-glutamate itself (Olney, 1969) and compounds activating EAA receptors coupled to ionophores have neurotoxic properties and can produce excitotoxic lesions which morphologically resemble human neurodegenerative disorders (Horowski et al., 1994). Under a variety of experimental conditions NMDA or glutamate mediated toxicity is blocked by competitive or non-competitive NMDA receptor antagonists such as 3-((\pm)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) or (+)-5-methyl-10,11-dihydro-5H-dibenzo-(a,d)cyclohepten-5,10-imine maleate (MK-801) *in-vitro* and *in-vivo*. Here we discuss experimental evidence linking MPTP or 1-methyl-4-phenylpyridinium ion (MPP⁺) mediated toxicity to EAAs.

Following systemic administration the neurotoxin MPTP produces a parkinsonian syndrome in humans and non-human primates (Davis et al., 1979; Langston et al., 1983; Burns et al., 1983). Similar to the observed histopathological features in Parkinson's disease (PD), dopaminergic neurons of the substantia nigra pars compacta (SNc) are primarily destroyed by the toxin (Burns et al., 1983), although other populations of cells are also affected to some degree (Pérez-Otaño et al., 1991). Thus, at least in primates, MPTP-induced neurodegeneration provides the animal model closest to PD that is currently available. However, there are histopathological and probably pathophysiological differences between the disease and the model situation in experimental animals. In addition, MPTP-induced cell death is an acute insult with symptoms occurring within hours to weeks whereas PD slowly progresses over years and some of the human histopathological features such as Lewy bodies, are rarely seen in brains of MPTP-treated primates (Forno et al., 1986). Nevertheless, the MPTP model in primates and, for practical reasons, in rodents offers the unique opportunity to study mechanisms involved in the destruction of dopaminergic neurons. A better understanding of the pathophysiology of MPTP could result in the development of strategies to protect cells and hopefully in preventive treatments of PD and other neurodegenerative diseases. Despite the intensive research in recent years the precise mechanisms mediating the neurotoxicity of MPTP

are not known although some crucial requirements are well established. MPTP readily passes the blood brain barrier. Probably in glial cells or non-dopaminergic neurons, MPTP is then oxidized to 1-methyl-4-phenyldihydropyridine (MPDP) which in turn is converted by monoamine oxidase type B (MAO-B) into the neurotoxic MPP⁺ (Castagnoli et al., 1985). The latter compound is accumulated in dopaminergic cells by the high affinity DA uptake carrier and temporarily stored in a releasable pool (Javitch et al., 1985; Schinelli et al., 1988). In addition MPP⁺ binds with high affinity to neuromelanin (D'Amato et al., 1986). Several mechanisms have been proposed to explain the toxicity at the cellular level. MPP⁺ is taken up intraneuronally by a mitochondrial carrier and inhibits complex I of the mitochondrial respiratory chain (Nicklas et al., 1985), resulting in a rapid cellular adenosinetriphosphate- (ATP) depletion (Chan et al., 1991) and subsequent cell death. This observation is of particular importance since an age-related reduction of complex I activity is observed in primates (Di Monte et al., 1993) and in post mortem brain tissue obtained from PD patients (Schapira et al., 1990). In addition, MPP⁺ could catalyse the formation of free radical species, resulting in oxidative cell damage (Lai et al., 1993) and MPP⁺ induces apoptosis (Dipasquale et al., 1991).

Experimental evidence suggests an involvement of EAAs such as glutamate within the neuropathological cascade of MPTP/MPP⁺-induced cell death. MPP⁺ induces in rats a release of glutamate and aspartate (Carboni et al., 1990) and a massive release of DA and lactate when delivered through a dialysis probe to the brain (Rollema et al., 1988). In addition we have shown that the neurotoxic properties of MPP⁺ following focal injection into the substantia nigra of rats are blocked by systemic treatment with non-competitive and competitive antagonist of NMDA receptors such as MK-801 or CPP, respectively (Turski et al., 1991). This report caused a controversial discussion with respect to the methods employed (Sonsalla et al., 1992). High local concentrations of MPP⁺ resulting from its focal administration to the brain could possibly produce unspecific toxic effects (Harik et al., 1987). It was therefore of importance to test whether this observation was restricted to the focal administration of MPP⁺ or whether it occurred following systemic treatment with MPTP. Since rats are not sensitive to MPTP, common marmosets were treated with MPTP alone and in combination with the competitive NMDA-antagonist CPP for up to two days. At post mortem the monoamine and metabolite content in the putamen were measured and a quantitative histological analysis of tyrosine hydroxylase (TH) positive neurons in the substantia nigra pars compacta was performed. MPTP-treatment resulted in a massive loss of intact TH-positive neurons in the substantia nigra, whereas CPP completely protected these cells. However, under the experimental conditions employed here, striatal levels of dopamine and its metabolites DOPAC and HVA were reduced (Lange et al., 1993). In line with these findings Zuddas et al. (1992) reported on the neuroprotective effects of MK-801 in another monkey species (*M. fascicularis*). Again, in animals treated with both MPTP and MK-801 nigral TH positive neurones were preserved, whereas striatal

dopamine and — to a larger extent — metabolite levels were reduced. Consistent with these findings we were unable to show a protective effect of MK-801 catecholamine levels in C57 black mice against systemic MPTP, employing repeated injection of the NMDA antagonist over two weeks and a prolonged survival time (Kupsch et al., 1992). These observations prompted us to study the effects of CPP upon MPTP toxicity in C57 black mice. In order not to interfere with the pharmacokinetic properties of both compounds we chose a survival time of four hours and post-mortem biochemical analysis of the striatum, cerebral cortex, brain stem (including the substantia nigra) and the cerebellum for DA, 3-methoxytyramine (3-MT), DOPAC and HVA as well as serotonin (5-HT) and 5-hydroxyindolacetic acid (HIAA) content.

Material and methods

Animals

Thirty-two male C57 black mice (Charles River Wiga, Germany), weighing 30–35 g and housed under standard conditions at a temperature of 22° ($\pm 1^\circ\text{C}$), 50% relative humidity and a 12 hour light-dark cycle (light on from 6.00–18.00 h) were allocated to four treatment groups.

Drugs and solutions

The following compounds were employed: CPP (3-((\pm)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, Research Biochemicals, USA), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride; Schering AG) dissolved in sterile physiological saline. All solutions were prepared immediately before administration and injected in a volume of 10 ml/kg body weight. All dosages refer to the free base.

Drug treatments and sample preparation

Groups of eight animals were treated with either vehicle or CPP intraperitoneally (25 mg/kg i.p.) 30 min prior to the intraperitoneal administration of MPTP (20 mg/kg i.p.) or vehicle. Four hours after administration of MPTP or vehicle the animals were sacrificed by decapitation. The brains were quickly removed and cut on an ice-cold plate into four regions: 1. the cerebral cortex ("cortex"), 2. the dopamine-rich part of the limbic system (olfactory tubercle, nucleus accumbens) and the striatum ("striatum"), 3. the mesencephalon and caudal regions ("brainstem") and 4. the cerebellum. Samples were weighed, immediately frozen on dry ice and stored at -80°C for biochemical analysis. Preparation of samples was performed within five minutes. All animal experiments were carried out in accordance with the recommendations of the Declaration of Helsinki and the animal welfare guidelines and laws of the Federal Republic of Germany.

Determination of biogenic amines

Frozen tissue samples were homogenized in 1 ml of 0.2N perchloric acid (10 ml containing 200 μ l of 10% EDTA and 100 μ l of 5% Na₂S₂O₅) and centrifuged at 30,000 g, 0°C for 15 min. Supernatants and diluted standard solutions were stored at -80°C for up to two weeks. Analysis of biogenic amines and metabolites was performed using high performance liquid chromatography (HPLC) with amperometric detection (electrochemical detector: Gynkotec M20, Pump: Gynkotec M 480, Injector: Gynkotec GINA 160, Germany) for simultaneous determination of dopamine, DOPAC (3,4-dihydroxyphenylacetic acid), HVA (homovanillic acid), 3-MT (3-methoxytyramine), 5-HT and HIAA. The oxidation potential was set at 690 mV and readings taken at 0.02 nA full scale. Striatal samples were diluted in aliquots of 0.2N perchloric acid immediately before injection to allow detection of dopamine under identical detector gain. Separation of amines was performed on a C18 reversed-phase column (Inertsil, 100 \times 2 mm, VDS Säulentechnik, Germany) in 10 μ l samples. The mobile phase consisted of 0.05 M sodiumphosphate buffer, pH 3.7, containing 250 mg octansulfonic acid and 3% isopropanol in deionized water (Millipore), the flow was set at 90 μ l/min. Quantification was performed on a 386 computer system using integration software (PE Nelson Turbochrome 2, USA) employing a three point external standard calibration curve. Raw amounts were corrected for dilution and expressed as amine content in pmol per mg wet tissue weight.

Statistical analysis

The mean \pm S.D. were calculated for monoamine concentrations of the four treatment groups in the four brain regions. Statistical differences between groups within one region were calculated by one-way analysis of variance (ANOVA) followed by post-hoc comparisons using the Tukey test (SYSTAT, USA).

Results

Administration of CPP resulted in a brief period of stereotyped behaviour followed by sedation and a mild hyperthermia. MPTP induced a stimulation of locomotor activity lasting 30 min and subsequently hypokinesia. Animals treated with CPP and MPTP were akinetic throughout the observation period.

The chromatographic system employed here allowed the simultaneous determination of dopamine and its principal metabolites DOPAC, HVA and 3-MT as well as 5-HT and its metabolite HIAA. Four hours after the administration of a single dose of MPTP (20 mg/kg i.p.) a more than 60% reduction of striatal dopamine and 80% of DOPAC, as compared to vehicle or CPP (25 mg/kg i.p.) treatment, was observed (cf. Table 1). Pretreatment with CPP in combination with MPTP resulted in a 40% reduction of striatal dopamine and 60% reduction in DOPAC concentrations. Levels of the two remaining dopamine metabolites HVA and 3-MT or the indolamines were unchanged. HVA and 3-MT concentrations were not affected in the brain stem. However, DOPAC tissue levels were reduced by 70–80% following treatment with MPTP plus CPP or CPP, respectively. MPTP alone reduced

Table 1. Effects of CPP 25 mg/kg i.p. (t = -30 min) and MPTP 20 mg/kg i.p. alone or in combination upon brain monoamine concentrations in C57 black mice

Region/Group	DA	DOPAC	HVA	3-MT	5-HT	HIAA
	[pmol/mg tissue wet weight, Mean (SD), N = 8]					
Striatum						
Vehicle	17.5 (3.1)	2.1 (0.4)	2.5 (1.2)	1.2 (0.5)	4.3 (0.8)	1.4 (0.2)
CPP	17.1 (3.1)	2.1 (0.4)	2.8 (1.4)	1.1 (0.6)	4.2 (0.8)	1.4 (0.4)
MPTP	6.5 (1.5) ^{a,b,c}	0.4 (0.2) ^{a,b}	2.5 (0.8)	1.0 (0.6)	4.3 (0.8)	1.1 (0.2)
CPP+MPTP	10.9 (2.5) ^{a,b}	0.9 (0.2) ^{a,b}	2.2 (1.2)	1.1 (0.3)	4.5 (0.7)	1.3 (0.2)
Brainstem						
Vehicle	0.6 (0.1)	0.3 (0.09)	0.5 (0.2)	0.07 (0.08)	7.3 (0.7)	3.4 (0.6)
CPP	0.7 (0.1)	0.4 (0.08)	0.6 (0.2)	0.06 (0.11)	7.5 (0.9)	4.1 (0.7)
MPTP	0.4 (0.1) ^{b,d}	0.05 (0.04) ^{a,b}	0.4 (0.2)	0.04 (0.02)	9.1 (1.0) ^d	3.5 (0.4)
CPP+MPTP	0.5 (0.1)	0.08 (0.05) ^{a,b}	0.5 (0.2)	0.06 (0.06)	9.5 (1.5) ^{a,b}	3.3 (0.8)
Cortex						
Vehicle	2.6 (0.4)	0.4 (0.1)	0.5 (0.2)	0.1 (0.1)	5.2 (0.6)	1.3 (0.3)
CPP	2.6 (0.3)	0.7 (0.1) ^a	0.7 (0.2)	0.1 (0.1)	5.0 (0.6)	1.6 (0.3)
MPTP	1.5 (0.2) ^{a,b}	0.1 (0.1) ^{a,b}	0.5 (0.1)	0.1 (0.1)	5.6 (0.5)	1.2 (0.2)
CPP+MPTP	1.9 (0.5) ^{a,b}	0.4 (0.1) ^{a,b}	0.6 (0.2)	0.2 (0.1)	6.1 (1.0) ^c	1.2 (0.3)
Cerebellum						
Vehicle	0.1 (0.04)	0.01 (0.02)	0.04 (0.06)	0.07 (0.2)	1.0 (0.3)	1.0 (0.2)
CPP	0.1 (0.06)	0.01 (0.02)	0.06 (0.05)	0.04 (0.1)	1.1 (0.4)	1.0 (0.3)
MPTP	0.1 (0.04)	n.d.	0.01 (0.03)	n.d.	1.4 (0.3)	0.7 (0.1) ^c
CPP+MPTP	0.1 (0.07)	n.d.	0.02 (0.05)	n.d.	1.6 (0.4)	0.7 (0.1) ^c

n.d. not detectable; ^a p < 0.01 vs vehicle; ^b p < 0.01 vs CPP; ^c p < 0.05 vs CPP plus MPTP; ^d p < 0.05 vs vehicle; ^e p < 0.05 vs CPP, ANOVA followed by the Tukey test

dopamine. Both treatments increased 5-HT concentrations by 20–30% in this brain region, whereas HIAA was not affected. Similar to the effects of both treatments upon striatal dopamine, dopamine and DOPAC levels were reduced in the cerebral cortex. CPP alone increased DOPAC and MPTP plus CPP increased 5-HT concentrations. There was no effect upon cerebellar dopamine. A marked decrease in metabolite concentrations occurred, so that DOPAC and 3-MT were not detectable in the MPTP and MPTP plus CPP groups. In addition HIAA levels were reduced whereas there was no effect upon 5-HT.

Discussion

The protective effects of NMDA antagonists in MPTP-induced neurodegeneration is still a matter of controversy. The present experiment was designed to elucidate acute effects of MPTP and the competitive NMDA antagonist CPP in black mice and to compare the present results with a study in common marmosets employing a similar treatment schedule of MPTP and CPP (Lange et al., 1993).

Within the terminal regions of DAergic innervation, i.e. the striatum and the cerebral cortex, MPTP induced a marked reduction in dopamine concentrations. This observation is compatible with the idea of intraneuronal MPP^+ uptake by nerve terminals and fibers but not by somata (Herkenham et al., 1991) via the dopamine transporter and subsequent inhibition of the mitochondrial respiratory chain. The reduced tissue concentration of DOPAC could also indicate inhibitory effects of MPP^+ upon monoamine oxidase (MAO) activity (Feuerstein et al., 1988), because this enzyme is located intraneuronally at mitochondrial membranes. The unchanged concentrations of 3-MT and HVA in both regions suggest that elimination of dopamine occurred without significant effects upon extraneuronal catecholamine metabolism since 3-MT is formed from dopamine by the extraneuronally located catechol-O-methyltransferase and subsequently oxidized by MAO to HVA. This effect of MPTP is only incompletely antagonized by pretreatment with CPP because both dopamine and DOPAC concentrations are significantly reduced in the group treated with CPP plus MPTP. In the brainstem preparation, containing the substantia nigra and the ventral tegment as well as the raphe nuclei, the results were different with dopamine being significantly reduced by MPTP-treatment alone and not affected in the group treated with MPTP and CPP. However, DOPAC levels were reduced in both groups. This could indicate that there is indeed a regional difference of the effects of MPTP upon catecholamine metabolism when competitive NMDA antagonists are present. Within the terminal regions CPP is only able to partially protect from dopamine depletion. While somata remain principally unaffected, there is a reduction in DOPAC formation. In line with this interpretation, we found in common marmosets variable effects of CPP upon MPTP-induced striatal catecholamine depletion but a survival of nigral tyrosinehydroxylase (TH) positive neurons

within the substantia nigra pars compacta at 48 hours following MPTP. Thus, these results provide additional evidence for the hypothesis that glutamate mediates neurotoxicity, at least at the level of nigral dopaminergic somata, and MPTP-induced cell death.

Although it is still not known how MPTP/MPP⁺ selectively destroys DAergic neurons, experimental evidence suggests the involvement of NMDA receptor-mediated events in this process. Due to impairment of mitochondrial respiration, MPP⁺ could induce a partial membrane depolarisation which in turn would remove the voltage-dependent Mg²⁺ block from NMDA receptor ion channels. This would enable EAAs such as glutamate to become neurotoxic at physiological concentrations through persistent receptor stimulation. The neurotoxicity of MPTP to DAergic terminals was not completely prevented by systemic treatment with CPP since the metabolite DOPAC was reduced in the striatum. At the level of DAergic somata, however, blockade of NMDA receptors prevents DA depletion. It may well be that two processes contribute to MPTP-induced neurotoxicity to dopaminergic cells: (i) direct impairment of respiration and metabolism in the terminals resulting in the well described retrograde degeneration of these cells (Kitt et al., 1987) and (ii) glutamate mediated excitotoxicity leading to destruction of the somata in the substantia nigra. Non-competitive or competitive NMDA antagonists such as MK-801 or CPP provide a certain degree of neuroprotection in various models in-vitro and in-vivo. However, there are complex pharmacokinetic and pharmacodynamic interactions between the toxin and putative protective agents which complicate the interpretation of results.

It should be kept in mind, however, that the term neuroprotection is used in many different ways. It usually applies only for the experimental conditions and/or the measures employed in a given experimental set-up. In animal experiments a neuroprotective agent should fulfil the following general criteria:

1. The measure to quantify the effect has to be relevant for the presumed clinical situation.
2. Protection of neurons from degeneration has to be shown at the morphological level. I.e. they should be indistinguishable from intact neurons when stained conventionally, immunohistochemically and using in-situ hybridisation.
3. Biochemical measures such as transmitter- and metabolite concentrations or enzymatic activities at the level of somata and the projection areas of the neurons should be unaffected by the toxin.
4. Behavioural observation of the experimental animals should show the normal repertoire of movements and higher skills.
5. The protective effect has to be permanent, i.e. survival times should control for both the chronic effect of the lesion and the presumed protective treatment.

When taking into account such strict criteria none of the published neuroprotective strategies, except inhibition of MAO to block formation of MPP⁺

(Heikkilä et al., 1984), seems to be effective in antagonizing MPTP-induced degeneration.

In conclusion, the protection from MPTP-mediated neuronal cell death, provided by NMDA antagonists, is restricted to nigral DAergic somata. Damage to cells occurs by retrograde axonal degeneration due to synaptic uptake of the toxin. Pharmacological interference with the pathophysiological events subsequent to the inhibition of mitochondrial respiration will very probably result in complete protection of the nigrostriatal system.

References

- Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, Kopin IJ (1983) A primate model of parkinsonism; selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc Natl Acad Sci USA* 80: 4546–4550
- Carboni S, Melis F, Pani L, Hadliconstantinou M, Rossetti Z (1990) The non-competitive NMDA-receptor antagonist MK-801 prevents the massive release of glutamate and aspartate from rat striatum induced by 1-methyl-4-phenylpyridinium (MPP⁺). *Neurosci Lett* 117: 129–133
- Castagnoli A Jr, Chiba K, Trevor AJ (1985) Potential bioactivation pathways for the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *Life Sci* 36: 225–230
- Chan P, DeLanny LE, Irwin I, Langston JW, Di Monte D (1991) Rapid ATP loss caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mouse brain. *J Neurochem* 57: 348–351
- Choi DW (1988) Glutamate toxicity and diseases of the nervous system. *Neuron* 1: 623–634
- D'Amato RJ, Lipman ZP, Snyder SH (1987) Selectivity of the parkinsonian neurotoxin MPTP: toxic metabolite MPP⁺ binds to neuromelanin. *Science* 231: 987–989
- Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, Kopin IJ (1979) Chronic parkinsonism secondary to intravenous injection of meperidine analogues. *Psychiatry Res* 1: 249–254
- Di Monte DA, Sandry MS, DeLanney LE, Jewell SA, Chan P, Irwin I, Langston W (1993) Age-dependent changes in mitochondrial energy production in striatum and cerebellum of the monkey brain. *Neurodegen* 2: 93–99
- Dipasquale B, Marini AM, Youle R (1991) Neuron apoptosis and DNA degradation induced by 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridinium (MPP⁺). *Biochem Biophys Res Commun* 181: 1442–1448
- Feuerstein TJ, Hedler L, Jackisch R, Hertting G (1988) An in vitro model of 1-methyl-4-phenyl-pyridinium (MPP⁺) toxicity: incubation of rabbit caudate nucleus slices with MPP⁺ followed by biochemical and functional analysis. *Br J Pharmacol* 95: 449–458
- Fonnum F (1984) Glutamate: a neurotransmitter in mammalian brain. *J Neurochem* 42: 1–11
- Forno LS, Langston JW, DeLanney LE, Irwin I, Ricaurte GA (1986) Locus ceruleus lesions and eosinophilic inclusions in MPTP-treated monkeys. *Ann Neurol* 20: 449–455
- Harik SI, Schmidley JW, Iacofano LA, Blue P, Arora PK, Sayre LM (1987) On the mechanism of underlying 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity: the effects of perinigral infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine its metabolite and their analogs in the rat. *J Pharmacol Exp Ther* 241: 669–676

- Heikkilä RE, Manzino L, Cabbat FS, Duvolsin R (1984) Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature* 311: 467–469
- Herkenham M, Little MD, Bankiewicz K, Yang SC, Markey SP, Johannessen JN (1991) Selective retention of MPP⁺ within the monoaminergic system of the primate brain following MPTP administration: an in vivo autoradiographic study. *Neuroscience* 40: 133–158
- Horowski R, Wachtel H, Turski L, Löschmann P-A (1994) Glutamate excitotoxicity as a possible pathogenetic mechanism in chronic neurodegeneration. In: Calne DB (ed) *Neurodegenerative diseases*. Saunders, Philadelphia, pp 163–175
- Javitch A, D'Amato RJ, Strittmatter SM, Snyder SH (1985) Parkinsonism inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity. *Proc Natl Acad Sci USA* 82: 2173–2177
- Johannessen JN, Chiueh CC, Burns RS, Markey SP (1985) Differences in metabolism of MPTP in the rodent and primate parallel differences in sensitivity to its neurotoxic effects. *Life Sci* 36: 219
- Kitt VA, Cork LC, Eidelberg E, Joh TH, Price DL (1987) Injury of catecholaminergic neurons after acute exposure to MPTA TH immunocytochemical study in monkeys. *Ann NY Acad Sci* 495: 730–731
- Kupsch A, Löschmann PA, Sauer H, Arnold G, Renner P, Pufal D, Burg M, Wachtel H, ten Bruggencate G, Oertel WH (1992) Do NMDA receptor antagonists protect against MPTP-toxicity? Biochemical and immunocytochemical analyses in black mice. *Brain Res* 502: 74–83
- Lai M, Griffiths H, Pall H, Williams A, Lunec J (1993) An investigation into the role of reactive oxygen species in the mechanism of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine toxicity using neuronal cell lines. *Biochem Pharmacol* 45: 927–933
- Lange KW, Löschmann PA, Sofic E, Burg M, Horowski R, Kalveram KT, Wachtel H, Riederer P (1993) The competitive NMDA antagonist CPP protects substantia nigra neurons from MPTP-induced degeneration in primates. *Naunyn Schmiedeberg's Arch Pharmacol* 348: 586–592
- Langston JW, Ballard B, Tetrud JW, Irwin I (1983) Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. *Science* 225: 1480–1482
- Nicklas WJ, Vyas I, Heikkilä RE (1985) Inhibition of NADH-linked oxidation in brain mitochondria by 1-methyl-4-phenylpyridine, a metabolite of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Life Sci* 36: 2503–2508
- Noveill A, Reilly JA, Lysko PG, Henneberry RC (1988) Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res* 451: 205–212
- Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurons. *Nature* 307: 462–465
- Olney JW (1969) Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science* 164: 719–721
- Olney JW (1989) Excitotoxicity and N-Methyl-D-aspartate receptors. *Drug Dev Res* 17: 299–319
- Olney JW, Labruyere J, Price MT (1989) Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs. *Science* 244: 1360–1362
- Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA (1991) NMDA antagonist neurotoxicity: mechanism and prevention. *Science* 254: 1515–1518
- Pérez-Otaño I, Herrero MT, Oset G, De Ceballos ML, Luquin MR, Obeso JA, Del Rio J (1991) Extensive loss of brain dopamine and serotonin induced by chronic administration of MPTP in the marmoset. *Brain Res* 567: 127–132
- Rollema H, Kuhr WG, Kranenborg G, DeVries J, Van den Berg C (1988) MPP⁺ induced efflux of dopamine and lactate from rat striatum have similar time courses as shown by in vivo brain dialysis. *J Pharmacol Exp Ther* 245: 858–866

- Schapira AHV, Mann VM, Cooper JM, Dexter D, Daniel SE, Jenner P, Clark JB, Marsden CD (1990) Anatomic and disease specificity of NADH CoQ₁ reductase (complex I) deficiency in Parkinson's disease. *J Neurochem* 55: 2142–2145
- Schinelli S, Zuddas A, Kopin IJ, Barker JL, di Prozio U (1988) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine and 1-methyl-4-phenylpyridinium uptake in dissociated cell cultures from embryonic mesencephalon. *J Neurochem* 50: 1900–1907
- Sonsalla PK, Zeevalk GD, Manzino L, Giovanni A, Nicklas WJ (1992) MK-801 fails to protect against the dopaminergic neuropathology produced by systemic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice or intranigral 1-methyl-4-phenylpyridinium in rats. *J Neurochem* 58: 1979–1982
- Turski L, Bressler K, Rettig KJ, Löschmann PA, Wachtel H (1991) Protection of substantia nigra from MPP⁺ neurotoxicity by N-methyl-D-aspartate antagonists. *Nature* 349: 414–418
- Watkins JC, Olverman HJ (1987) Agonists and antagonists for excitatory amino acid receptors. *TINS* 10: 265–280
- Zuddas A, Oberto G, Vaglini F, Fascetti F, Fornai F, Corsini GU (1992) MK-801 prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in primates. *J Neurochem* 59: 733–739

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