THE CANADIAN JOURNAL OF NEUROLOGICAL SCIENCES

The Neurotoxicity of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the Monkey and Man

R. Stanley Burns, Sanford P. Markey, Jere M. Phillips and Chuang C. Chiueh

ABSTRACT: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) selectively destroys dopaminergic neurons in the pars compacta of the substantia nigra (A_8 and A_9 cells). MPTP or its metabolite enters nerve cells at the level of their terminals in the caudate nucleus and putamen leading to a disturbance in axoplasmic flow and retrograde degeneration. The species-dependent neurotoxicity of MPTP (primate vs. rodent) suggests that a biochemical property of the cell related to neuromelanin may be important in the mechanism of cell injury.

RÉSUMÉ: Le MPTP détruit sélectivement les neurones dopaminergiques de la pars compacta de la substance noire (cellules A8 et A9). Le MPTP ou son métabolite pénètre dans les cellules au niveau des terminaux dans le noyau caudé ou putamen et conduit à une interférence dans le flot axoplasmique et une dégénérescence rétrograde. La dépendance de cet effet à la race animale (primate *vs* rongeur), suggère que la neuromélanine joue un rôle important dans le mécanisme de l'atteinte cellulaire.

Can. J. Neurol. Sci. 1984; 11:166-168

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, or NMPTP) selectively destroys dopaminergic neurons in the substantia nigra and produces a parkinson-like syndrome in man (Davis et al., 1979; Langston et al., 1983) and in non-human primates (Burns et al., 1983) which is similar to idiopathic Parkinson's disease in its clinical, pathological, biochemical and pharmacological response features. It is unlikely that MPTP is the single chemical in the universe that can produce this toxic effect. The great degree of similarity between MPTP-induced parkinsonism in man and idiopathic Parkinson's disease suggests that it may represent more than a model. The occurrence of MPTP-induced parkinsonism leads one to consider a toxic cause for Parkinson's disease.

Several toxic agents that produce damage to component nuclei of the basal ganglia in primates resulting in extrapyramidal disorders have been identified. The mechanisms of action of the chemical agents (6-hydroxydopamine, MPTP, possibly manganese) with selective toxic effects on catecholaminergic neurons (Burns et al., 1983; Chandra et al., 1979; Gupta et al., 1980; Neff et al., 1969; Sachs and Jonsson, 1975) are of greater interest than the agents (carbon monoxide, carbon disulfide) which produce focal areas of necrosis in the region of the globus pallidus (Klawans et al., 1982; Richter, 1945). Understanding the mechanism of action of MPTP may increase our knowledge of the pathogenesis of Parkinson's disease.

Toxic Effects in the Monkey

Intravenous administration of MPTP (a cumulative dose of 1.7 mg/kg) to non-human primates (Macaca mulatta, Macaca fascicularis, Saimiri sciurea) produces a chronic parkinson-like disorder (akinesia, rigidity, postural tremor, a flexed posture, impaired righting reflexes, drooling) that is reversed by L-dopa or lisuride (unpublished observations). Rhesus monkeys fail to exhibit motor abnormalities until multiple doses of MPTP have been administered and the concentration of homovanillic acid in ventricular cerebrospinal fluid, a measure of dopamine release, has fallen to about 30% to 40% of the normal level indicating a large functional reserve in the system. The degree of motor impairment resulting from this weight adjusted dose of MPTP is variable from animal to animal. Older monkeys (10-12 yr.) appear to be more vulnerable to the toxic effects of MPTP than juvenile monkeys, although we have not performed a systematic study of the influence of age.

MPTP selectively and irreversibly damages dopaminergic neurons in the pars compacta of the substantia nigra (corresponding to areas A8 and A9 of the rat brain) of the rhesus monkey leading to a severe loss of pigmented neurons in this nucleus (Figure 1) and a marked reduction in the dopamine

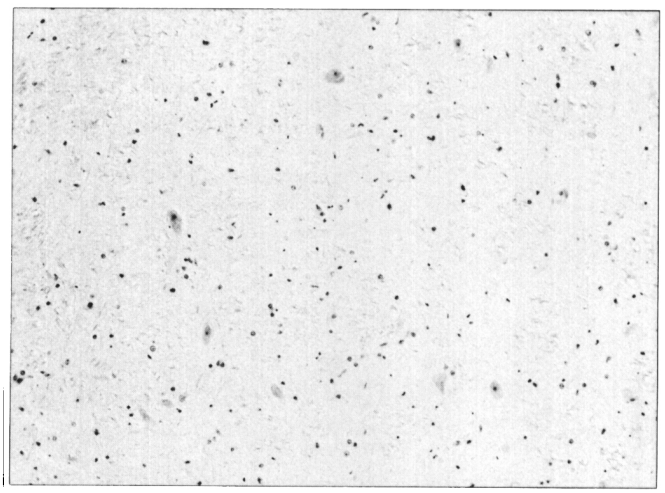

Figure 1— Transverse section through the midbrain showing the substantia nigra of an MPTP-treated monkey. Note severe nerve cell loss. (Hematoxylin/eosin stain; X130.)

From the Section on Experimental Therapeutics (Dr. Burns), Section on Analytical Biochemistry (Dr. Markey), Laboratory of Clinical Sciences, and the Section on Laboratory Animal Medicine and Care (Dr. Phillips), National Institute of Mental Health, Bethesda, Maryland.

Reprint requests to: R. Stanley Burns, M.D., SET, LCS, NIMH, Bldg. 10, Rm. 3S231, 9000 Rockville Pike, Bethesda, Maryland 20205 U.S.A.

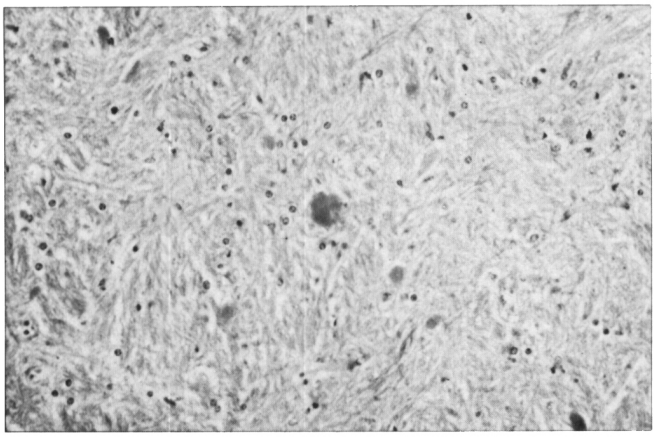

Figure 2 — Transverse section through the midbrain of an MPTP-treated monkey showing the region of the substantia nigra. Note the presence of large (10-20 + μ), eosinophilic bodies representing swollen axons. (Hematoxylin/eosin stain; X400.)

content of the striatum. The dopaminergic neurons in the ventral midbrain (corresponding to area A10 of the rat brain) appear normal on histofluorescent staining and the dopamine content of their terminal regions, i.e. nucleus accumbens, olfactory tubercule, is normal. Dopaminergic neurons in the median eminence, and noradrenergic neurons in the locus ceruleus and hypothalamus also appear normal. Regional dissection as well as autoradiographic studies of $^3H_2$-MPTP show that at 24 hours the radiolabel (MPTP and/or metabolites) is widely distributed throughout the grey matter of the brain with the highest densities in the regions of the caudate nucleus, putamen and nucleus accumbens (unpublished observations), suggesting that MPTP enters nerve cells of the substantia nigra at the level of their terminals in the striatum. Histofluorescence studies reveal the accumulation of dopamine within swollen axons immediately above the substantia nigra at 1 month after treatment with MPTP. On light microscopy these swollen axons appear as large (10-20μ) eosinophilic bodies in the region of the proximal axon (Figure 2).

The neurotoxicity of MPTP is species dependent. Primates exhibit akinesia which is accompanied by a decrease in the dopamine content of the striatum and nerve cell loss in the substantia nigra following intravenous administration of MPTP. These changes do not occur in the rat (Chiueh et al., 1984) or guinea pig (unpublished observations). The pathological changes in the rhesus monkey, cynomologus monkey and man are similar despite differences in exposure to MPTP (Davis et al., 1979; unpublished observations).

Toxic Effects in Man

Several individuals developed persistent symptoms of parkinsonism following intravenous self-administration of multiple doses of an illicit analgesic preparation containing a potent analog of meperidine and a synthetic side product, MPTP (Davis et al., 1979; Langston et al., 1983). MPTP was shown to be the neurotoxic agent responsible by reproducing the neurological syndrome in the rhesus monkey (Burns et al., 1983). Patients with MPTP-induced parkinsonism exhibit a pure extrapyramidal syndrome with hypokinesia, rigidity, a resting tremor, a flexed posture, loss of postural reflexes, hypomimia, drooling, speech disturbance and a positive Glabellar relfex (unpublished observations). Dementia, autonomic impairment, cerebellar signs or pyramidal tract signs are not observed. All of the clinical signs are reversed by the administration of L-dopa (combined with carbidopa), bromocriptine, or lisuride.

A severe loss of pigmented neurons in the substantia nigra with minimal changes in the locus ceruleus was found in the one patient who died of other causes 18 months after the onset of symptoms (Davis et al., 1979). Biochemical studies in four patients with MPTP-induced parkinsonism revealed low levels of homovanillic acid, the major metabolite of dopamine, in lumbar cerebrospinal fluid (unpublished observations). The concentrations of 3-methoxy-4-hydroxyphenylethylene glycol and 5-hydroxyindolacetic acid, the major metabolites of norepinephrine and serotonin, respectively, were normal or mildly increased.

All of the major clinical features of idiopathic Parkinson's disease are present in the syndrome of MPTP-induced parkinsonism; they can be specifically attributed to degeneration of the nigrostriatal pathway and a deficiency of dopamine in the striatum. The pathological changes in MPTP-induced parkinsonism are limited to the substantia nigra and do not include other pigmented nuclei of the brainstem which are consistently involved in idiopathic Parkinson's disease, i.e. locus ceruleus, dorsal motor nucleus of vagus. The formation of Lewy body inclusions which is characteristic of idiopathic Parkinson's disease has not been satisfactorily demonstrated in man or the monkey following exposure to MPTP.

Mechanism of Action

MPTP is a small, lipid-soluble molecule which rapidly diffuses into brain tissue following intravenous administration and, most likely, enters nerve cells of the substantia nigra at the level of their terminals in the caudate nucleus and putamen. Although the biochemical mechanism of injury to the cell is unknown, the initial accumulation of axoplasm and dopamine within the proximal axon followed by cell loss is consistent with a disturbance of axoplasmic flow and retrogade degeneration.

MPTP destroys dopaminergic neurons in the substantia nigra (A9 cells) which terminate in the putamen, whereas dopaminergic neurons in close proximity in the ventral midbrain (A10 cells) terminating in the nucleus accumbens adjacent to the putamen are left intact. Spatial-volume characteristics of the cells, accumulation via the dopamine uptake mechanism and the absence of the enzyme dopamine-ß-hydroxylase (found in unaffected noradrenergic neurons) do not explain the selective effect on a subgroup of proximal dopaminergic neurons. It is unknown whether at higher doses of MPTP other dopaminergic or even noradrenergic neurons might be affected.

Some biochemical property of the dopaminergic neurons in the substantia nigra (A8 and A9 cells) makes them differentially susceptible to the toxic effects of MPTP. The species dependent neurotoxicity of MPTP (primates vs. rodents) and the factor of age parallel the presence and accumulation of neuromelamin in dopaminergic neurons in the substantia nigra of primates (Barden, 1981; Barden and Levine, 1983). Non-enzymatic autoxidation of dopamine results in products which polymerize to generate neuromelanin (Barden, 1981; Graham, 1978). The cytotoxicity of 6-hydroxydopamine is thought to arise from the formation of O_2^-, H_2O_2, and $^{\bullet}HO$ via autoxidation, (Graham et al., 1978) and that of manganese from the enhanced autoxidation of dopamine (Donaldson et al., 1982). If the toxicity of MPTP similarly involves an oxidative mechanism, then differences in

the accumulation of MPTP (or its metabolites) or in the content of protective enzymes, e.g. superoxide dismutase, glutathione peroxidase, catalase, may explain the selective effect on a subgroup of dopaminergic neurons (Ambani et al., 1975; Ledig et al., 1982).

References

Ambani LM, Van Woert, MH, Murphy S (1975) Brain peroxidase and catalase in Parkinson disease. Arch. Neurol. 32: 114-118.

Barden H (1981) The biology and chemistry of neuromelanin. In *Age Pigments*, Sohal RS (Ed), 155-180, Elsevier/North-Holland Biomedical Press, Amsterdam.

Barden H, Levine S (1983) Histochemical observations on rodent brain melanin. Brain Res. Bull. 10: 847-851.

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.

Chandra SV, Srivastava RS, Shukla GS (1979) Regional distribution of metals and biogenic amines in the brain of monkeys exposed to manganese. Toxicology Lett. 4: 189-192.

Chiueh CC, Markey SP, Burns RS, Johannessen JN, Pert A, Kopin IJ (1984) Neurochemical and behavioral effects of systemic and intranigral administration of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the rat. Eur. J. Pharmacol. (Accepted).

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.

Donaldson J, McGregor D, Labella F (1982) Manganese neurotoxicity: a model for free radical mediated neurodegeneration? Can. J. Physiol. Pharmacol. 60: 1393-1405.

Graham DG (1978) Oxidatiue pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. Mol. Pharmacol. 14: 633-643.

Graham DG, Tiffany SM, Bell WR, Gutknecht (1978) Autoxidation verses covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxdopamine, and related compounds toward C 1300 neuroblastoma cells in vitro. Mol. Pharmacol. 14: 644-653.

Gupta SK, Murthy RC, Chandra SV (1980) Neuromelanin in manganese-exposed primates. Toxicology Lett. 6: 17-20.

Klawans HL, Stein RW, Tanner CM, Goetz CG (1982) A pure parkinsonian syndrome following acute carbon monoxide intoxication. Arch Neurol. 39: 302-304.

Langston JW, Ballard P, Tetrud JW, Irwin I (1983) Chronic parkinsonism in humans due to a product of meperidine-analog synthesis. Science. 219: 979-980.

Ledig M, Fried R, Ziessel M, Mandel P (1982) Regional distribution of superoxide dismutase in rat brain during postnatal development. Developmental Brain Research. 4: 333-337.

Neff NH, Barrett RE, Costa E (1969) Selective depletion of caudate nucleus dopamine and serotonin during chronic manganese dioxide administration to squirrel monkeys. Experientia. 25: 1140-1141.

Richter R (1945) Degeneration of the basal ganglia in monkeys from chronic carbon disulfide poisoning. J. Neuropath. Exp. Neurol. 4: 324-353.

Sachs C, Jonsson G (1975) Mechanisms of action of 6-hydroxydopamine. Biochem. Pharmacol. 24: 1-8.