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Paradoxical sleep deprivatory effect of a single low dose of MPTP which did not produce dopaminergic cell loss

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Abstract. In *a* previous study, we reported on a selective and long-lasting paradoxical sleep (PS) deprivation in cats following *repeated* administration of the Parkinson syndrome-inducing neurotoxin *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). While the characteristic motor deficits occurred only from the 2nd to 3rdday of a 5-day long administration of 5 mg/kg per day MPTP i.p., the PS deprivation started immediately after the first injection and lasted altogether for 11–13 days. The motor deficiencies induced by *repeated* administration of MPTP are mainly due to the selective depletion of dopaminergic neurons in the nigrostriatal system as the histological and biochemical data show. The immediate onset of PS deprivation and the PS recovery, despite the definite cell loss, suggests a mechanism independent of cell destruction. In our present study we investigated the occasional histological and the PS-deprivatory effect of a single low dose of MPTP in cats. A single injection of 2 mg/kg MPTP i.p. resulted in PS deprivation lasting for 2.5–3.5 h. The duration of other sleep stages showed no significant change and PS recovery was without rebound phenomenon, *as in the case of repeated administration.* Even a higher single dose of MPTP (5 mg/kg) resulted in no visibly detectable nigrostriatal cell loss. We suggest that the changes in monoamine release and/or turnover are involved in the PS deprivatory effect of MPTP.

Key words: MPTP – Paradoxical sleep – Cat

Introduction

Our previous study disclosed a selective and long-lasting paradoxical sleep (PS) deprivation in cats during *repeated* administration of the Parkinson syndrome-inducing neurotoxin *N*–methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Pungor et al. 1990). PS deprivation started immediately after the first injection, lasted through the 5-day-long administration of 5 mg/kg per day MPTP i.p. and for a further 6–7 days following the last injection. PS recovery was parallel to the recovery of the motor activity. However, the parkinsonian-like changes in motor activity occurred only after the 2nd–3rd day of repeated MPTP administration. Nevertheless, we observed some immediate vegetative phenomena (pupillary dilatation, hypersalivation, diarrhoea), after each injection, lasting for 20–60 min.

The characteristic long-lasting changes in motor activity (e.g. difficulties in initiation of movements, decrease in total amount of movements) are mainly due to the selective destruction of dopaminergic neurons in the nigrostriatal system, as the histological and biochemical data show (Chiueh et al. 1984; Kopin 1987; Langston 1985; Schneider et al. 1986). The immediate onset of PS deprivation, the immediate vegetative symptoms and the PS recovery in spite of definite cell loss in chronic experiments suggests a mechanism independent of cell destruction. Therefore, in the present study we investigated the short-term effect of a single low dose of MPTP on the sleep-wakefulness cycle and its occasional cytotoxic effect in the dopaminergic cell areas in cats.

Materials and methods

Experiments in freely moving cats

Experiments were carried out in four adult cats (2.4–3.3 kg). Under pentobarbital sodium (Nembutal) anaesthesia (40 mg/kg), cats were implanted with electrodes for electroencephalography (EEG), electromyography (EMG) and electro-oculography (EOG). Gold-plated screw electrodes were inserted symmetrically into the skull above right and left somatosensory, associative, temporal and occipital cortices to record EEG. Two stainless steel electrodes were placed into the neck muscles for EMG and a further two screw electrodes into the left frontal cavity for EOG. To habituate the cats to their experimental environment they were placed individually in soundproof, electrically shielded recording cages 1 week before implantation. On the basis of the visual evaluation of the polygraphic records and computerized analysis of the EEG with a CED 1401 EEG analysis system, the sleep-wakefulness cycle was divided

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into wakefulness (W), slow-wave-sleep light phase (SWS1), characterized by sleep spindles and fluctuation of synchronization in delta frequency range (0.2–2.0 Hz), slow-wave-sleep deep phase (SWSd), dominated by delta waves, and into PS. The criteria of PS was the disappearance of muscular tone in the neck, the presence of rapid eye movements in the EOG, EEG desynchronization and occurrence of ponto-geniculo-occipital (PGO) waves in the occipito-occipital lead (Jones 1991). The relative amount of each sleep stage per hour was determined. A two-tailed Student's *t*-test was used for statistical analysis.

Recording was carried out between 9 a.m. and 1 p.m. Control recording sessions were performed following a 3- to 5-day-long recovery period after the implantation. Control recordings lasted for at least 3 days, during which 2 ml of physiological saline was injected i.p. immediately before starting the recording. Following control experiments, a single dose of 2 mg/kg MPTP dissolved in 2 ml physiological saline was injected i.p. Sleep recording was continued for a further 2 days after MPTP administration to test the amount of PS.

Histological studies

To compare the cytotoxic effects of a single MPTP dose and that of repeated administration of this toxin we used two groups of cats. Three animals were treated with a single, but higher, dose of MPTP (5 mg/kg i.p. dissolved in 2 ml sterile physiological saline) than in the sleep experiments. Two cats were given a 5 mg/kg/day dosage of MPTP (each dissolved in 2 ml of sterile physiological saline) for 6 consecutive days. Each of these five cats was anaesthetized, 1 week after MPTP treatment, with Nembutal, and perfused with buffered saline (pH 7) and with fixation solution (10% formaldehyde pH 7), then the brains were removed. After 2 weeks of fixation, the brains were embedded in paraffin. 5–5, 20-µm serial sections were made from the cerebral cortex, the striatum, the diencephalon, the mesencephalon, the pons and the medulla oblongata and stained using the Klüver-Barrera method. After visual evaluation of slides, photographs at different magnifications were taken of substantia nigra to estimate the dopaminergic cell loss.

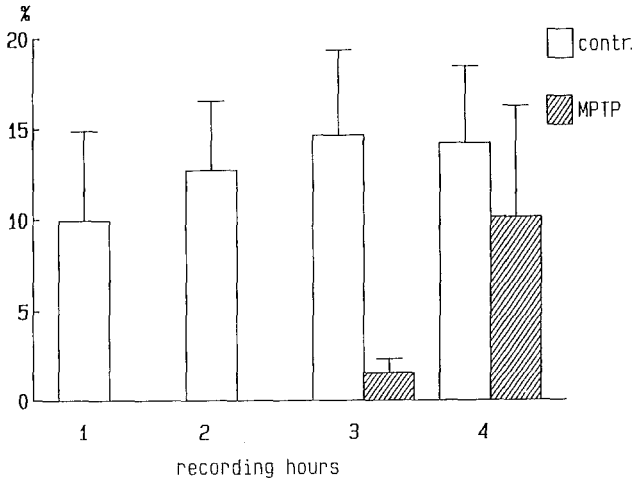

Fig. 1. The means of the relative amount (percentage) of paradoxical sleep (PS) per hour during the first 4-h-long registration following injection of a single low dose (2 mg/kg) of MPTP i.p. in cats ($n=4$; *striped column*) compared with control *(open column)*. PS is totally suppressed during the first 2 h, appears at the 3rd h, and no significant difference could be observed at the 4th h

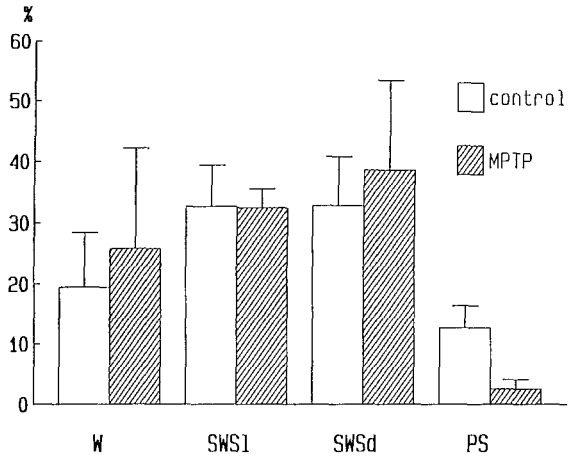

Fig. 2. The means of the relative amount (percentage) of the four sleep stages in total sleep time: wakefulness (*W*), slow-wave-sleep light phase (*SWSl*), slow-wave-sleep deep phase (*SWSd*) and paradoxical sleep (*PS*) in control period *(open column)* and after MPTP treatment with 2 mg/kg MPTP i.p. (striped column) in cats ($n=4$). There was no significant change in the duration of W, SWSl, SWSd. The low amount of PS is the result of PS reappearance in the 3rd h of registration after the MPTP treatment (2 mg/kg)

Results

Effects of 2 mg/kg MPTP on sleep

The single dose of 2 mg/kg MPTP resulted in PS deprivation lasting for 2.5–3.5 h in each cat. There was no significant difference in the relative amount of PS in the 4th h following MPTP administration, though it was slightly decreased (Fig. 1), and no rebound phenomena were observed throughout the 2 days after MPTP treatment. There was no significant change in the relative amount of SWSl, SWSd and W in MPTP-treated animals compared with control recordings (Fig. 2). The four cats showed no change in motor performance and there was no vegetative reaction (hypersalivation, pupillary dilatation, diarrhoea) after the injection of 2 mg/kg MPTP.

Anatomical findings

Administration of single doses of 5 mg/kg MPTP for 6 consecutive days resulted in a marked reduction in number of neurons and glial proliferation in the substantia nigra of cats (Fig. 3C). One dose of 5 mg/kg MPTP, however, did not induce visibly detectable cell loss (Fig. 3B) in the substantia nigra, estimated on the basis of counting cell numbers in ten visual fields from control and MPTP-treated animals. Finer degenerations in neurons, however, cannot be excluded by visual evaluation.

Discussion

Our present findings clearly demonstrate that a single low dose (2 mg/kg i.p.) of MPTP is able to influence PS-generating mechanisms. The MPTP-induced PS de-

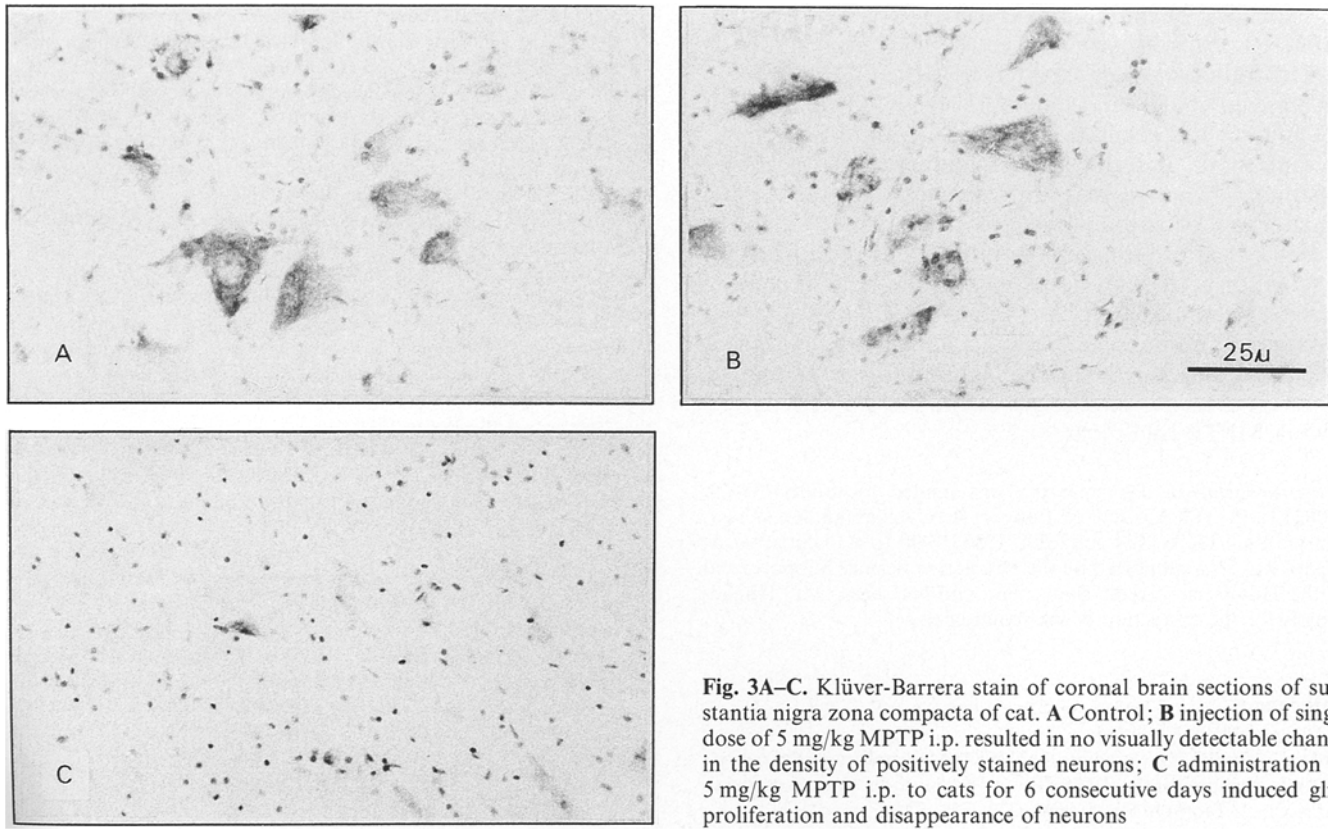

Fig. 3A–C. Klüver-Barrera stain of coronal brain sections of substantia nigra zona compacta of cat. **A** Control; **B** injection of single dose of 5 mg/kg MPTP i.p. resulted in no visually detectable change in the density of positively stained neurons; **C** administration of 5 mg/kg MPTP i.p. to cats for 6 consecutive days induced glial proliferation and disappearance of neurons

privation occurs at lower doses as well, but the duration of this effect seems to be dose-dependent, since in our previous experiments with repeated doses, the PS deprivatory effect of the first injection of 5 mg/kg MPTP was continuous till the following day's MPTP administration (after 24 h; Pungor et al. 1990). The lack of acute vegetative signs at low doses of MPTP suggests a different background of these two phenomena (i.e. PS deprivation and vegetative symptoms).

The PS deprivatory effect of MPTP seems to be independent also from the MPTP-induced parkinsonian-like movement disorder of cats. As was demonstrated in our previous studies with repeated administration of MPTP, a long-lasting PS deprivation during the 5-day treatment period, and for an additional 6–7 days after the last dose, could be achieved. The recovery of PS was parallel with the recovery of the motor functions, but the alterations of the motor performance occurred only 2–3 days after beginning the chronic MPTP treatment, which suggested the independence of motor deficiency from PS deprivation to some extent (Pungor et al. 1990). This observation was confirmed in our present study: a low dose of MPTP was able to induce PS deprivation without any definitive motor deficiency.

In our earlier studies, PS generation recovered in spite of the marked reduction of monoaminergic cells in the brain stem (Pungor et al. 1990), indicating that PS deprivation by MPTP could not be based on the monoaminergic cell loss. The experimental evidence presented here clearly demonstrates that the death of doparminergic cells is not necessary for PS deprivation induced by MPTP. 2 mg/kg MPTP sufficed to deprive PS, but an even higher dose (5 mg/kg) of MPTP failed to induce a marked deficit in catecholaminergic neurons in a single dose.

The death of dopaminergic cells seems to be the result of intracellular action of 1-methyl-4-phenylpyridinium (MPP^+), formed from MPTP by monoamine oxidase B (Chiba et al. 1984; Poirier 1987). MPP^+ is a free radical which is taken up by the neurons preferentially not only through the dopamine uptake site (Herkenham et al. 1991; Irwin and Langston 1985; Jawitch and Snyder 1985; Langston et al. 1984; Singer and Ramsay 1990) but also on other monoamine uptake sites (Kopin 1987; Langston 1985). One of the major actions of MPP^+ is the modification of the cytochrome system (Langston and Irwin 1986; Nicklas et al. 1985). In chronic experiments (after 3–6 dosages of 5 mg/kg MPTP injected i.p. in cats on consecutive days) this inhibition of the respiratory chain results in dopaminergic cell death and leads to parkinsonian signs when it reaches a critical level (Schneider et al. 1986). In turn, even a small dose of MPTP could decrease monoamine metabolic rate, as supported by experimental evidence (Chiueh et al. 1984; Markey and Schmuff 1986; Schneider et al. 1986). Thus, we assume that the MPTP-induced decrease in monoamine availability might be in causal relation to the MPTP-induced PS deprivation. This seems to be supported by the idea that control of cyclic alterations of sleep stages is regulated by reciprocal interaction of acetylcholinergic and monoaminergic neurons of the brain stem. However, in this reciprocal-interaction model, PS

is assumed to be initiated by the fall in activity of monoaminergic neurons near to zero (Hobson et al. 1986). It makes it difficult to understand how the MPTP-induced decrease in monoamine metabolism could deplete but not initiate the generation of PS. On the basis of our present work and the quoted literature on the acute monoamine changes after MPTP treatment, the normal functioning of monoaminergic neurons seems to be necessary for initiating PS. Another putative cause of PS deprivation after MPTP treatment could be a decrease in cholinergic activity (Hobson et al. 1986; Jones 1991) of pedunculopontine tegmental nuclei (PPN). However, this remains only a theoretical assumption, since there is no direct evidence of changes in cholinergic activity following MPTP treatment.

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References

Chiba K, Trevor AJ, Castagnoli N (1984) Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. Biochem Biophys Res Comm 120:574–578

Chiueh CC, Markey SP, Burns RS, Johannessen JN, Pert A, Kopin IJ (1984) Neurochemical and behavioral effect of systemic and intranigral administration of *N*-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine in the rat. Eur J Pharmacol 100:189–194

Herkenham M, Little MD, Bankiewicz K, Yang SC, Johannessen JN (1991) Selective retention of MPP^+ within the monoaminergic systems of the primate brain following MPTP administration: an in vivo autoradiographic study. Neuroscience 40:133–158

Hobson JA, Lydic R, Baghdoyan KA (1986) Evolving concepts of sleep cycle generation: from brain centers to neuronal populations. Behav Brain Sci 9:371–448

Irwin I, Langston JW (1985) Selective accumulation of MPP^+ in the substantia nigra: a key to neurotoxicity? Life Sci 36:207–212

Jawitch JA, Snyder SH (1985) Uptake of MPP^+ by dopamine neurons explains selectivity of parkinsonism inducing neurotoxin MPTP. Eur J Pharmacol 106:455–456

Jones BE (1991) Paradoxical sleep and its structural/chemical substrates in the brain. Neuroscience 40:637–656

Kopin IJ (1987) MPTP: an industrial chemical and contaminant of illicit narcotics stimulates a new area in research on Parkinson's disease. Environ Health Perspect 75:45–51

Langston JW (1985) MPTP neurotoxicity: an overview and characterization of phases of toxicity. Life Sci 36:201–206

Langston JW, Irwin I (1986) MPTP: current concepts and controversies. Clin Neuropharmacol 9:485–507

Langston JW, Irwin I, Langston EB, Forno L (1984) 1-methyl-4-phenylpyridinium ion (MPP^+): identification of a metabolite of MPTP, a toxin selective to the substantia nigra. Neurosci Lett 214:1370–1372

Markey SP, Schmuff NR (1986) The pharmacology of the parkinsonian syndrome producing neurotoxin MPTP (1-methyl-4-phenyl-1,2,3-6-tetrahydropyridine) and structurally related compounds. Med Res Rev 6:389–429

Nicklas WJ, Vyas I, Heikkila 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

Poirier J (1987) Pathophysiology and biochemical mechanisms involved in MPTP-induced parkinsonism. J Am Geriatr Soc 35:660–668

Pungor K, Papp M, Kékesi K, Juhász G (1990) A novel effect of MPTP: the selective deprivation of paradoxical sleep in cats. Brain Res 525:310–314

Schneider JS, Yuwiler A, Markham CH (1986) Production of a Parkins-like syndrome in the cat with *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): behavior, histology and biochemistry. Exp Neurol 91:293–307

Singer TP, Ramsay RR (1990) Mechanism of the neurotoxicity of MPTP. An update. FEBS Lett 274:1–8