計劃名稱：氧化氮在 MPTP 神經毒理作用中扮演的角色

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中文摘要

已知 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine（MPTP）是一種特殊的神經毒素，能夠在人類及靈長類誘發酷似巴金森氏病（巴病）的病理及臨床變化。過去我們已證明 MPP^+ 能夠在大鼠的紋狀體造成巴多胺之異常釋放 dopamine depletion），產生羥基自由基(hydroxyl radical)及脂質過氧化物。最近有報告指出一種氧化氮合成酵素（nitric oxide synthase，NOS）抑制劑 7-nitroindazol（7－NI）能夠預防 MPTP 之毒性。MPTP 的毒性代謝產物 MPP^+ 能夠干擾粒腺體電子傳遞系統，產生自由基 superoxide anion 經由活化 N-methyl-D-aspartate（NMDA）接受體而產生的 $NO^{\bullet}$ 能夠與 superoxide 結合形成自由基 peroxynitrite；peroxynitrite 進一步分解形成 $OH^{\bullet}$ 自由基而對細胞產生傷害。然而亦有研究發現另一種 NOS 抑制劑 L-N^G nitro arginine methyl ester（L－NAME）卻無法在一種中南美洲小猿猴（marmoset）身上對抗 MPTP 之毒性。本研究利用顱內微透析法（microdialysis procedure）進一步探討 NOS 抑制劑，包括 7-NI 及 L-NAME 對 MPP^+ 在紋狀體內所誘發的多巴胺之過度溢流，多巴胺的代謝產物及形成羥基自由基之影響，結果發現兩種藥物都無法保護神經細胞對抗 MPP^+ 之毒性。

關鍵詞：MPTP, nitric oxide, Parkinsonism, 7-nitroindazole, L-N^G nitro arginine methyl ester, microdialysis

Abstract

methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to produce parkinsonism in humans and monkeys. Our previous studies have shown that MPP^+ induced dopamine depletion and hydroxyl radicals formation and lipid peroxidation in the striatum of rats. Recent reports have suggested that MPTP toxicity could be prevented by 7-nitroindazole (7-NI, a potent inhibitors of neuronal nitric oxide synthase). It is suggested that MPP^+ may interfere with mitochondrial electron transport and result in a leakage of superoxide anion. Nitric oxide ($NO^{\bullet}$), produced following activation of N-methyl-D-aspartate (NMDA) receptors, combined with superoxide to form the toxic free radical peroxynitrite, which in turn may degenerate into more noxious hydroxyl radical and cause cell damage. However, evidence suggested that another nitric oxide synthase (NOS) inhibitor, L-N^G nitro arginine methyl ester (L-NAME, another NOS inhibitor) did not show protection against MPTP-induced toxicity in the marmoset. Using the MPTP-Parkinsonism model, the present study showed that both of 7-NI and L-NAME did not exert protective on

dopaminergic neurons in term of the inhibition of dopamine depletion and hydroxyl radical formation induced by MPP^+ in the striatum.

Introduction

The discovery of MPTP, a specific neurotoxin can selectively destroy midbrain dopaminergic neurons and produces a parkinsonian syndrome in both human and monkey, has led to the extensive studied in elucidating the biochemical mechanisms underlying its neurotoxicity. Recent evidence suggests that an exicitotoxic mechanism may play a role in the pathogenesis of Parkinson's disease. It has been demonstrated that MPTP toxicity could be prevented by 7-nitroindarole (7-NI), a potent inhibitor of neuronal nitric oxide synthase (nNOS) (Babbedge et al., 1993, Schulz et al., 1995, Moore and Bland-Wad, 1996; Przedborski et al., 1996, Hasntraye et al., 1996). Przedborski et al. (1996) also showed that mutant mice lacking the NOS gene were more resistant to MPTP than the wild-type littlemates. This finding further linked nitric oxide (NO°) formation to MPTP-induced toxicity. However, the findings that another nitric oxide synthase (NOS) inhibitor, L-N^G nitro arginine methyl ester (L-NAME, another NOS inhibitor) did not show protection against MPTP-induced toxicity in the marmoset. Moreover, NMDA receptors blocker have not been proven to exert consistent neuroprotection against MPTP toxicity. Thus, the role of NO° in the nigrostriatal toxicity induced by MPTP remains to be clarified.

Using a modified microdialysis procedure, we have recently demonstrated that intrastriatal infusion of MPP^+ evoked dopamine depletion and formation of hydroxyl radicals as reflected by salicylate hydroxylation in rats (Chiueh et al., 1992, 1993; Wu et al., 1993). In the present study, the role of NO° in the formation of hydroxyl radicals induced by
MPP+ will be investigated by using NOS inhibitors, 7-NI and L-N^G nitro arginine methyl ester.

Materials and Methods

Male Sprague-Dawley rats (250-350 g) are anesthetized with chloral hydrate (400 mg/kg, i.p.) and prepared for intracranial microdialysis brain perfusion. MPP^+, salicylate (Sigma, St. Louis, MO) and 7-NI , are dissolved peant oil (Sigma) for s.c. injection. L-NAME (RBI) was dissolved in sterile saline. A microdialysis probe (Carnegie Medicine CMA/12, Sweden) is stereotaxically implanted into striatum in one cerebral hemisphere (stereotaxic coordinates: AP 10, R/L:2.5, H:3; Paxinos and Watson, 1982). The striatum is then perfusned with Ringer's solution (1 ml/min) through the probe. Following a 100 min washout with Ringer's solution, MPP^+ is infused (5 nmol/ml/min for 15 min; total dose: 75 nmol) to induce dopamine overflow. Salicylate is subsequently perfused (5 nmol/ml/min for 75 min) to trap hydroxyl radical generated in the extracellular fluid. Using a similar within-subjects design, some animals receive salicylate infusion (5 nmol/ml/min for 75 min) alone following 75 min washout with Ringer's solution into the striatum on one side to obtain a perfusion reagent baseline. On the contralateral striatum in the same subject, MPP^+ and salicylate are perfused as described above. However, a single s.c. injection of different doses of 7-NI and L-NAME (25 mg/kg and 50 mg/kg) was administrated to produce maximal inhibition of NOS. Brain dialysate is collected at 15 min interval

into 15 ml 0.1% Na_2EDTA in 0.1 N $HClO_4$ and immediately assayed for 2,3-dihydroxybenzoic acid, dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) by an HPLC-EC procedure .

Results and Discussion

Our results found that either pretreatment with 7-NI (25 and 50 mg/kg,s.c.) or L-Name (25 mg/kg,s.c.) did not offer protection on the dopaminergic neurons against the injury induced by MPP+. Fig 1 showed intrastriatal perfusion of MPP^+ (75 nmol; 5 mM, 1uL/min for 15 min) through microdialysis probe induced a significant increase of extracellular levels of dopamine (Fig 1a) and 2,3-dihyroxylbenzoic acid (2,3-DHBA), the hydroxyl radical adduct of salicylate over perfusion reagent baseline (Fig1b). Pretreatment with 7-NI (25mg/kg, s.c.) one hour before the infusion of MPP^+ did not provide effect on the results induced by MPP^+ in the striatum (Table 1). Fig 2a and b demonstrated the result of pretretment with L-Name (25 mg/kg, s.c.). The data were shown in the table 1. The present results suggested that $NO^\bullet$ may not contribute to the free radical formation induced by MPP+ in the striatum.

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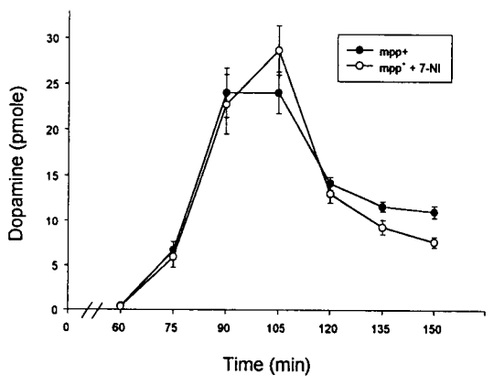
Table 1. The effect of NO inhibitors (7-NI, L-NAME, 25mg/kg,s.c.) on the dopamine efflux and 2,3 DHBA formation induced by MPP^+.

(N)	DA (pmole)	DC (pmole)	2,3DHBA (pmole)
MPP^+ (7)	106.2±10.6	114.9±13.2	24.4±4.3
MPP^+ + 7-NI (7)	89.1±9.9	93.2±8.3	24.6±2.5
MPP^+ + L-NAME (8)	116.6±15.1	94.4±11.8	21.5±3.2

Fig.1

(a)



(b)

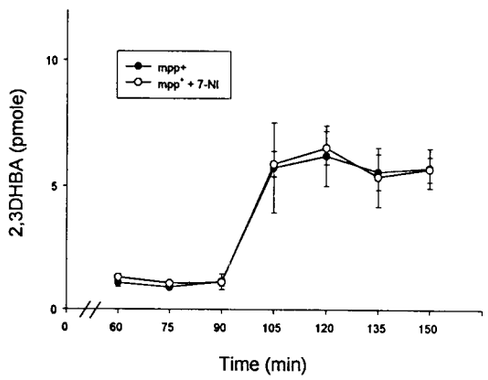
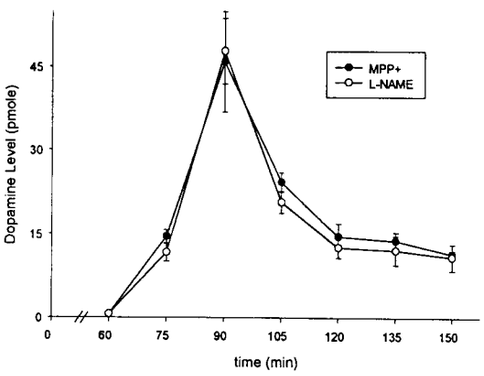


Fig.2

(a)



(b)