P02.571 EFFECTS OF NICOTINE ON 1-METHYL-4-PHENYL-PYRIDINIUM UPTAKE IN PC12 CELLS AND SK-N-SH CELLS

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Statement of the study: Evidences from epidemiological studies suggest a negative correlation between cigarette smoking and the occurrence of Parkinson's disease. It has been demonstrated that nicotine administration or cigarette smoking partially prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine(MPTP), a parkinsonism-inducing neurotoxin, -induced nigrostriatal degeneration. However, the effects of nicotine on uptake of 1-methyl-4-phenylpyridinium ion (MPP+), which is a major toxic metabolite of MPTP, into the cells are not fully understood. In the present study to ascertain the influence of nicotine on the transporter activity, we examined the effects of nicotine on the uptake of MPP+ in rat pheochromocytoma PC12 cells and human neuroblastoma SK-N-SH cells where norepinephrine transporter is functionally expressed.

Methods: To assay MPP+ uptake, cells were washed with Krebs-Ringer-HEPES (KRH) buffer and then incubated with 5 nM [^{3}H]MPP+ in KRH buffer for 10 min. After incubation, cells were washed with ice cold KRH buffer and then radioactivity remaining in the cells was extracted and measured by the liquid scintillation counter.

Summary of results: Nicotine or acetylcholine (ACh) significantly suppressed [^{3}H]MPP+ uptake in a concentration-dependent manner. Epibatidine, a potent agonist for neuronal nicotinic ACh receptor, also had similar effect on [^{3}H]MPP+ uptake. Inhibition of [^{3}H]MPP+ uptake by nicotine or ACh was fully reversed by hexamethonium, but not by ⟨-bungarotoxin, an α7 nicotinic acetylcholine receptor-selective antagonist. The inhibitory actions of nicotine or ACh were not prevented by removing extracellular Ca2+ or blocking the voltage-dependent calcium channel by nifedipine. Moreover, calphostin C, an inhibitor of protein kinase C, had no effect on the nicotine-suppressed [^{3}H]MPP+ uptake. Gramicidin, a Na+ ionophore, reduced the [^{3}H]MPP+ uptake. In contrast, decrease of extracellular Na+ concentration suppressed the inhibitory actions of nicotine. These results suggest that the decrease in Na+ gradient across the plasma membrane produced by nicotine led to the down-regulation of the transporter activity. Furthermore, we investigated the effect of nicotine on MPP+-induced cell death in PC12 cells. Pretreatment of PC12 cells with nicotine significantly prevented the following MPP+-induced cell death in a concentration-dependent fashion.

Conclusion: Taken together, it is suggested that depolarization-dependent suppression of MPP+ influx triggered by nicotine is the crucial mechanism for the protection against MPP+ toxicity.