THE NEUROTOXICITY OF INSECTICIDES TO STRIATAL DOPAMINERGIC PATHWAY

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ABSTRACT

Parkinson's disease (PD) is an age-related neurodegenerative disease, which is characterized by severe loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and consequent dopamine depletion in its projecting area. In this dissertation, I evaluated the neurotoxicity of several classes of insecticides/drugs/neurotoxins to the striatal dopaminergic pathway and their potential relationship to Parkinsonism in the C57BL/6 mouse model, using biochemical and molecular biology methods.

In the first objective, I investigated the neurotoxicity in striatal dopaminergic pathways following co-application of permethrin (PM), chlorpyrifos (CPF) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The study was done because pyrethroid and organophosphorus compounds are widely used insecticides and they have been implicated in Gulf War Syndrome. We found that short-term, high-dose exposure to PM or CPF had no significant effects on the expression of dopamine transporter (DAT), tyrosine hydroxylase (TH), or α-synuclein protein in striatal nerve terminals, but the insecticides slightly enhanced the neurotoxicity of MPTP in C57BL/6 mice at 28 days post-treatment. This finding indicates a slowly developing neurotoxicity may occur after termination of high-dose exposure. Long-term, low-dose exposure to PM did not show significant neurotoxicity to striatal dopaminergic pathways when given alone, nor did this injection of PM enhance the neutotoxicity of MPTP in C57BL/6 mice. In addition,

experiments with pure *cis* or *trans* isomers of permethrin showed that both *cis* and *trans* isomers contributed equally to the neurotoxicity of PM in the short-term high dose study.

Previous studies demonstrated a deficiency in mitochondrial function in PD, and a high density of K^+_{ATP} channels are present in substantia nigra, which play an important role in the maintenance of the membrane potential under metabolic stress. Therefore, in the second objective, I investigated the effect of K^+_{ATP} channel blockage on the neurotoxicity of mitochondrial-directed neurotoxins to striatal dopaminergic pathways. I found that mitochondrial inhibitors are potent releasers of preloaded dopamine from striatal nerve terminals, with the most potent compounds active in the nanomolar range. Co-application of the K^+_{ATP} channel blocker glibenclamide selectively increased the dopamine-releasing effect by complex I inhibitors *in vitro*, and potentiated the neurotoxicity of MPTP (a complex I inhibitor) on DAT and TH expression, *in vivo*. Mechanistic studies demonstrated that mitochondrial inhibitor-induced dopamine release is Ca^{2+}-dependent. In addition, the selectivity of glibenclamide is not correlated to ATP depletion, but associated with the generation of excessive reactive oxygen species at the site of complex I.

In the third objective, I conducted comparative studies on the mode of action of rotenone-/reserpine-/tetrabenzaine (TBZ)-induced neurotransmitter depletion, *in vitro*, as these three compounds share some similarities in their chemical structures. I found that rotenone, reserpine and TBZ selectively released preloaded dopamine and serotonin (5-HT), with the rank order as rotenone>reserpine>TBZ. Mechanistic studies demonstrated more than one mechanism was involved in both rotenone- and reserpine-induced neurotransmitter release. Ca^{2+}-stimulated vesicular release and neurotransmitter

transporter-mediated release are the common mechanisms involved in rotenone- and reserpine-induced dopamine release.

Overall, the insecticides/drugs/neurotoxins tested in the above experiments all exhibited some effect on the nigrastrital dopaminergic pathway, either alone or by enhancing the toxicity of other chemicals in combination treatment.