

Chapter 8 Summary and conclusions

8.1 MAO-A and MAO-B active sites

One of the goals of this project was to investigate the active sites of MAO-A and MAO-B using designed bulky substrates to probe the steric limits in the active sites as there are no x-ray structures of these mitochondrial membrane bound enzymes. With the designed C4 substituted naphthyl and biphenyl series we were able to define regions of activity and inactivity for the A and B forms of MAO. These results show clearly that the MAO-A and MAO-B active sites differ in volume in different regions. These results also tend to indicate that the MAO-A active site is not larger than the MAO-B active site as previously thought.^{148,149} We were also able to expand upon these topological activity analyses to develop updated active site models for MAO-A and MAO-B that allow us to begin to explain some of the selectivities observed with various tetrahydropyridinyl substrates.

Additional effort focused on the design of series of MPTP derivatives bearing polar functionalities. The results have allowed us, with the aid of molecular modeling, to establish a topological relationships between MPTP derivatives and endogenous substrates and to map the areas of the active sites that can accommodate polar functionalities.

8.2 MAO catalyzed neurotoxicity in mice

Another aim of this work was to investigate the mechanism of MAO-A mediated neurotoxicity in vivo as well as trying to establish a relationship between the observed in vitro substrate activity and in vivo enzyme mediated neurotoxicity. We were very fortunate to obtain one of the most selective MAO-A substrate reported, 1-methyl-4-(2-phenylphenyl)-1,2,3,6-tetrahydropyridine.²⁸⁵ This selective A substrate was used to evaluate MAO-A mediated neurotoxicity in mice. The results indicate that the *o*-biphenyl tetrahydropyridinyl substrate displays MAO-A mediated neurotoxicity in vivo. This analog, however, was not an ideal A substrate because it was not very water soluble and displayed other general toxicities which were not identified but led to the death of the animals. In our lab we are currently searching for more water soluble tetrahydropyridines that display MAO-A selectivity as predicted by our active site models.

It would appear that there should be a direct and definable relationship between high in vitro substrate activity and neurotoxicity, but this is not the case. Included in the panel of tetrahydropyridines examined in vivo was 1-methyl-4-(3-methylfuran-2-yl)-1,2,3,6-tetrahydropyridine, which is one of the best MAO-B substrates obtained (approximately 5.3 times more active than MPTP). At doses twice those of MPTP only half of the toxic response in C57Bl/6 mice was observed. From the investigation of various C4 substituted tetrahydropyridines in vivo, we must conclude that there are several factors involved in eliciting an MAO mediated neurotoxic response, that it may be difficult to develop simple relationships between activity with the pure enzyme and in vitro neurotoxicity.

Factors that may be involved include lipophilicity of the compound, which controls its diffusion through membranes, how much of the unchanged compound reaches the brain and the levels, and inherent toxicities of the pyridinium metabolite. All these factors must be measured and controlled to evaluate the relationship between neurotoxicity and substrate properties.

8.3 Evaluation of 7-NI neuroprotection

The role of MAO in neurodegenerative diseases has been recognized for several years. Agents that protect against MAO mediated neurotoxicity may have pharmaceutical value in the treatment of Alzheimer's and Parkinson's disease, neurodegenerative diseases for which there is currently no cure. It is well established that MPTP produces a selective neurotoxicity that is MAO-B mediated. Using the MPTP model of neurotoxicity we were able to evaluate the role of the reported selective nNOS inhibitor 7-NI on MPTP metabolism and neurotoxicity in vivo. Contrary to previously reported results,²²⁸ we were able to demonstrate that 7-NI is a competitive inhibitor of MAO-B in vivo and is not an exclusive nNOS inhibitor. 7-NI was reported to display neuroprotection against MPTP mediated neurotoxicity as a result of its inhibition of nNOS.²²⁷ Our work clearly indicates that 7-NI, being an inhibitor of MAO-B, slows the metabolism of MPTP to its metabolites in the striatum of C57Bl/6 mice which is a contributing factor to the neuroprotection observed against MPTP toxicity.