MASTER'S THESIS

Modulation of N-methyl-D-aspartate receptor expression in neuronal cell culture

Lui, Pik Wa

Date of Award:
2002

Modulation of N-methyl-D-aspartate Receptor Expression in Neuronal Cell Culture

LUI Pik Wa

A thesis submitted in fulfillment of the requirements

for the degree of

Master of Philosophy

Principal Supervisor: Dr. YUNG Kin Lam

Hong Kong Baptist University

December 2002

Abstract

N-methyl-D-aspartate (NMDA) receptors are known to play important roles in neuronal growth and development as well as in glutamate excitocytotoxicity that results in neuronal cell death. Excitocytotoxic cell death is suggested to be one of the major causes of human neurological diseases. NMDA receptors are ionotropic glutamate receptors and they are composed of heteromeric subunits (NR1, NR2A-D and NR3). A functional NMDA receptor is known to be composed of NR1 subunit coupled to at least one form of NR2 subunits. The functional properties of the NMDA receptors depend on their subunit compositions. NMDA receptors are one of the most important groups of glutamate receptors in neurons of the rat basal ganglia, a group of subcortical nuclei that is associated with movement. The neostriatum and the substantia nigra are two principal regions of the basal ganglia. Neuronal cell death in these two regions is known to cause Huntington's and Parkinson's disease respectively.

In the first part of the present study, developmental expression and functional properties of NMDA receptors in cultured striatal and nigral neurons were studied. Both striatal and nigral neurons were found to express NR1 mRNA and immunoreactivity at different time frames. In addition, striatal neurons were found to express NR1, NR2A, NR2B and NR2D mRNA and immunoreactivity whereas nigral neurons were found to express NR1, NR2B and NR2D. Patch clamp experiments revealed that only the striatal neurons but not the nigral neurons in culture displayed functional NMDA channels. Striatal neurons but not the nigral neurons were also found to respond to NMDA-induced excitocytotoxicity. These results indicate that different types of neurons of the basal ganglia display different intrinsic programs of

NMDA receptor expressions that may form NMDA receptors with different functional properties.

Based on the above results, striatal neurons were selected as a model to study the effects of modulation of gene expression of NMDA receptor subunit. The second part of the present study was to investigate the aftermath effects on NMDA receptor expression and functional properties after selective gene knockdown of NR1 subunit in the striatal neurons in culture. Striatal neurons were treated with a single dose of antisense oligonucleotides specific for NR1 (ANR1), sense oligonucleotides specific for NR1 or a randomized sequence based on NR1 sequence. Decreases in expression of NR1 mRNA and proteins were found only after ANR1 treatments but not in the other control treatments. There was no modification of the NR2 subunit expression after ANR1 application. Interestingly, application of ANR1 was found to provide neuroprotective effects to the striatal neurons against NMDA-induced excitocytotoxicity. Moreover, NMDA channel-mediated peak inward currents were found to be reduced after the application of ANR1. The present data suggest that ANR1 is an effective and useful tool in performing selective gene knockdown of NR1 subunit in striatal neurons. As a potential neuroprotective agent, ANR1 may be developed into a potent agent for treatment of neurological diseases.

Table of Contents