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AMPLIFICATION AND CLONING OF NR3 REGION OF NMDA RECEPTOR SUBUNIT

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ABSTRACT

This thesis focused on the amplification and verification of NR3 subunits of the excitatory glutamate receptor, ionotropic N-methyl-D-aspartate. The functional unit of cation channel gated is believed to be a tetrametric complex structure which consists of NR1, NR2 and NR3 subunits. The glycine binding to NR3 subunit is required for the opening of the ion channel. We cloned the mice NR3A and NR3B which has only minor differences in homology to rat NR3 subunit. The RNA was extracted from adult albino mice brain tissue and converted into cDNA by using two steps Reverse-Transcriptase Polymerase Chain Reaction. The NR3A and NR3B were amplified by using specific primer which has been synthesized based on previous studies. The NR3A and NR3B were constructed into pcDNA and pTARGET respectively and cloned into E.coli. Both plasmid then were extracted from E.coli and purified before they were sent for sequencing and verification by using NCBI. Only NR3B was successfully constructed into pTARGET showing specific region from residue TM1 until S2. Eventhough NR3A was not successfully constructed into pCDNA but the NR3A PCR product was blasted and attributed to specific region C-terminal phosphorylation site.

CHAPTER 1

INTRODUCTION

1.1 Background study

Both *in vivo* and *in vitro* studies of NMDA have provided a lot of information on the basic properties of the NMDA receptor, such as the ion permeation and gating properties including deactivation and desensitization. *In vitro* studies of NMDA receptor has a few disadvantages where the transfection of NR1, NR2 or NR3 alone into the cell is useless because the NMDA receptor works in complement of each other for activation (Gereau & Swanson, 2008). *In vivo* studies of recombinant gene construct of NMDA receptor produce results that are inconsistent. It is also a problem to use recombinant methods in heterologous cells to interpret desensitization mechanism of native receptors. Since the *in vivo* study of NMDA receptors is problematic, it is not commonly used by researchers (Gereau & Swanson, 2008). However, the result of *in vivo* studies is more accurate than *in vitro* studies. In order to closely understand the *in vivo* function of the NMDA receptor, dissociated cultures are used where the native receptors are examined.