UNIVERSITI TEKNOLOGI MARA

THE NEUROPROTECTIVE MECHANISM OF DREAM VIA ERAD PATHWAY IN DYHYDROXYPHENYLGLYCINE PRECONDITIONED ACUTE ISCHEMIC STROKE RATS

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Neuroprotective strategies are required to complement the available medical treatments in order to enhance the brain endogenous protective mechanisms and cushion the effect of stroke injury. Pharmacological preconditioning is an avenue of preventative medication anticipated to be highly effective in protecting and reducing the ischemic induced neuronal damage. Recently, *in vitro* preconditioning studies have shown that prior activation of group I metabotropic receptor (mGluR) with its specific agonist (S)-3,5-dihydroxyphenylglycine ((S)-3,5-DHPG) elicits neuroprotection against excitotoxicity. Furthermore, the activation of group I mGluR regulates the expression of DREAM. DREAM protein regulates transcription of various genes including *edem1* which is a component protein of ER-associated degradation pathway (ERAD). This study elucidates the neuroprotective effect of group I mGluR agonist preconditioning, (S)-3,5-DHPG via DREAM and ERAD in acute ischemic stroke rats. One, 10 or 100 μM (S)-3,5-DHPG was administered intrathecally to 6 adult male Sprague Dawley rats 2 hours prior to the middle cerebral artery occlusion. After 24 hours, the modified neurological severity score (mNSS) and grid walking test were assessed. The rats were sacrificed and the infarct brain volumes were estimated by 2,3,5-triphenyltetrazolium chloride staining. The serum level of neuron-specific enolase (NSE) and brain tissue level of Bip/GRP78 ER stress marker were assessed by ELISA assays. The ischemic penumbra tissue surrounding the ischemic core infarct was dissected and the cytoplasmic and nuclear proteins as well as the total RNA were extracted. The protein levels of nuclear and cytoplasmic DREAM, as well as EDEM1, SEC61α and VCP were analysed by Western blot. The expression of *dream* and *edem1* genes were analysed by qRT-PCR. Finally, the level of protein degradation activity in the ischemic penumbra tissue was determined by the 20S proteasomal assay. One or 10 μM of (S)-3,5-DHPG preconditioning in stroke rats has significantly improved the neurological functions and reduced the brain infarction as well as the NSE level. The DREAM protein has significantly increased in the nuclear compartment after 2 hours of 1 μM (S)-3,5-DHPG administration and in the cytoplasmic compartment after 24 hours of 100 μM (S)-3,5-DHPG administration. Similarly, 1 μM (S)-3,5-DHPG preconditioning has significantly reduced the levels of Bip/GRP78 ER stress marker, DREAM and ERAD proteins as well as proteasomal degradation activity after 24 hours of an ischemic stroke. The expression of *dream* and *edem1* gene were decreased in 1 μM (S)-3,5-DHPG preconditioning compared to non-preconditioning ischemic stroke rats. In conclusion, the 1 and 10 μM of (S)-3,5-DHPG preconditioning enhanced the endogenous protective mechanism via promoting the nuclear DREAM protein to regulate the expression of EDEM1 and ERAD activities in order to alleviate subsequent ischemic injury in the brain whereas 100 μM of (S)-3,5-DHPG preconditioning exacerbated the ischemic injury.

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