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Hippocampal Endoplasmic Reticulum Stress: Novel Target in PTSD Pharmacotherapy?

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Posttraumatic stress disorder (PTSD) is an anxiety disorder that occurred in individual who had experienced severe traumatic stresses. This disorder is accompanied by functional impairments in daily activities, comorbidities (such as depression) and increased risk of suicide. Some studies also demonstrate that PTSD is linked to structural and functional impairment of hippocampus. Hippocampal defect has been found in PTSD model, especially in single-prolonged stress (SPS)-induced animal model, with excessive or prolonged endoplasmic reticulum (ER) stress-induced neuronal apoptosis as a proposed mechanism. Unfortunately, this cellular event has not been studied and validated in humans suffering from PTSD. Two chaperones known as glucose-regulated protein 78 (GRP78) and sigma-1 receptor (Sig1R) have been demonstrated to exhibit central roles in mitigating the effects of severe ER stress on cell survival. Several selective serotonin-reuptake inhibitors (SSRIs), such as fluvoxamine and sertraline, are also found to be an agonist and antagonist of sigma-1 receptor (Sig1R) in animal brain cells, respectively. There is also link between antidepressant use and risk of suicidal ideation. Therefore, the authors propose that hippocampal ER stress may be involved in PTSD pathobiology. Pharmacodynamics of currently available therapeutic agents for PTSD and its comorbidities on hippocampal ER stress should be clearly elucidated to promote therapy optimization and drug development.

Keywords: ER stress, hippocampus, GRP78, Sigma-1 receptor, PTSD, therapy.

Posttraumatic stress disorder (PTSD), a potentially debilitating mental condition, affects many people in the world. PTSD is an anxiety disorder that occurred in individuals exposed to dramatic stresses (e.g., death threats, severe traumatic injuries)[1, 2]. According to diagnostic criteria in the Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV), PTSD patients show characteristic clinical features, especially flashbacks of traumatic memories, and subsequent intense fear and/or sense of helplessness[2]. These patients may also show avoidances to flashback-evoking stimuli, emotional numbness, and hyperaroused state[3]. This disorder is accompanied by functional impairments in daily activities and increased risk of suicide[4].

PTSD can be viewed as psychological stress that can physically alter brain structure and

function. Patients with PTSD are known to have smaller hippocampal volumes compared to those of normal control[2]. Intriguingly, several recent studies point out that animal model of PTSD is linked to endoplasmic reticulum (ER) stress, especially apoptosis of neuronal cells in hippocampus[2, 5]. Sertraline, a potent selective serotonin reuptake inhibitor (SSRI) drug approved for PTSD pharmacotherapy, is known to be associated with enhanced oxidative stress in brain, spatial memory impairment and reduced hippocampal glucose-regulated protein 78 (GRP78) expression[6, 7]. GRP78, a highly conserved cytoprotective protein, is the master regulator of unfolded protein response (UPR)[8, 9]. UPR is primarily activated by cell to mitigate the potentially harmful effects of ER stress on cell survival[10]. It is also known that ER stress is tightly connected with oxidative stress[11], thus the administration of SSRI, especially sertraline, may exacerbate ER stress by enhancing oxidative stress. Hayashi proposed that during psychological stress cellular chaperone such as sigma-1 receptor (Sig1R) also hold cytoprotective roles in stressed neuronal cells[12]. Therefore, under the light shed by studies or papers on GRP78 and Sig1R, the authors propose that hippocampal ER stress is an interesting and novel potential target of PTSD pharmacotherapy.

The Involvement of Hippocampus in PTSD

Brain has the capacity to adapt to environmental stimuli such as chronic stress. Resilience and neuroplasticity, two brain adaptive mechanisms to stress, can be facilitated by hippocampus[13]. Hippocampus (consists of gyrus dentatus, cornu ammonis (CA1-CA3), and subiculum) plays important roles in facilitating long term memory in other cortical areas of the brain[14]. Hippocampus is also involved in encoding, recognition of episodic memory, and environmental cues (contexts)[3]. Hippocampus, that has important roles in the establishment of spatial and long term memory, expresses all families of serotonin receptor (5-HT). Thus, hippocampus serves important functions in both cognitive and affective domains[13, 15]. Interestingly, hippocampus is recently known to be actively involved in language processing, especially in associating percepted words to semantic memory[16].

Glucocorticoid production may attenuate hippocampal neurons excitability, whereas 5-HT_{1A} receptor activation by serotonin may protect neurons from lowered excitability[17]. Excessive glucocorticoid production, in combination with persistant hypothalamus-pituitary-adrenal (HPA) axis activation, can directly decrease hippocampal serotonin level. These events lead to the elevation of fear intensity and depressive mood, and to the reduction of resilience. Hippocampus also expresses significant number of norepinephrine (NE) receptors. The activation of this receptor during stress event may also contribute to the reinforcement of long-term memory[18]. These findings may be viewed as the basis of traumatic stress-related memory persistence.

Based on animal studies, hippocampal impairment may be linked to PTSD, due to its susceptibility to psychological stress, and probably, endoplasmic reticulum (ER) stress[2, 5]. Hossain *et al*. also showed that learning deficits could be associated with hippocampal ER stress, at least in adult mice[19]. Cognitive deficits and memory dysfunctions can be commonly found in PTSD cases[1]. Other study showed that the severity of hippocampal functional impairment was correlated with the severity of PTSD sign and symptoms[20].

ER Stress and PTSD: Roles of GRP78 and Sig1R

Mammalian cells exposed to various stressors may accumulate unfolded proteins and misfolded proteins inside the ER lumen[21, 22]. This condition is known as ER stress. This type of stress activates unfolded protein response (UPR) as a cellular mechanism to prevent or ameliorate negative effects of the stressors[21]. The UPR serves to restore protein homeostasis within ER. The induction of UPR triggers intracellular signaling pathway to communicate the presence of unfolded and misfolded proteins within ER lumen to nucleus and cytoplasm. Accumulation of unfolded and misfolded proteins into insoluble aggregates may become the pathogenetic basis of various diseases[22].

Major regulator of UPR is an ER chaperone called glucose-regulated protein-78 (GRP78), also known as immunoglobulin heavy chain-binding protein (BiP). This protein works by binding to unfolded and misfolded proteins[21, 23]. GRP78 also regulates ER stress transducers. Upregulation of GRP78 has been used as an UPR onset biological marker[21]. Besides its chaperoning activity, GRP78 is also involved in the targeting of

misfolded proteins to proteasomal degradation[23]. Thus, if there are accumulations of unfolded and misfolded proteins, GRP78 expression can be upregulated to prevent proteotoxic injury[24].

Excessive ER stress may cause intrinsic apoptotic pathway activation and subsequent cell death[25]. Hence, GRP78 also plays role as an ER stress-related apoptosis regulator. This role is established via binding-release mechanism of ER stress transducers. ER stress transducers or sensors include ATF6, IRE1, and PERK. These transducers are also known as "The Three Arms of UPR". When the cell is unstressed, all of these transducers are under inactive state. In the presence of unfolded and misfolded protein accumulation, GRP78 will disassociate with the luminal domains of ER stress transducers. This disassociation will trigger a network of molecular interactions that determines cell fate, whether it will undergo apoptosis or not[26]. As an example, if ER stress occurs, GRP78 overexpression will inhibit procaspase-7 and procaspase-12 activation. The inhibition of these procaspases will subsequently lead to the inhibition of proapoptotic proteins (Bik and Bax, for examples) activation and prevent mitochondrial release of c-cytochrome[23]. These events contribute to cell survival via the inhibition of intrinsic apoptotic pathway activation.

Han *et al*. found that there is decrease in GRP78 expression, increase in caspase-12 expression and the number of apoptotic cells in hippocampus of single-prolonged stress (SPS)-induced rodent model of PTSD. The SPS protocol involves 3 types of stress such as restraining for 2 hours (psychological stressor), forced swim (physical stressor), and exposure to ether anaesthesia (biochemical stressor)[2]. The combination of these stressors has been found to produce model that mimic PTSD pathophysiology in human, while also reducing the risk of habituation in the model[3]. Unfortunately, to date, the exact role or nature of association of GRP78 in PTSD pathogenesis and pathophysiology is unknown[2]. Other studies revealed that activation of caspase-12 is known as an ER stress-specific cellular event[27, 28]. These findings point out that caspase-12 activation is a key signal in failure of ER stress mitigation and further strengthens GRP78's role as antiapoptotic molecule. These findings lead to the assumption that there is some degree of correlation between ER stress, apoptosis, and hippocampal defect in animal model of PTSD. We speculate that the correlation may also exist in PTSD patients and demands thorough investigations of PTSD pathobiology focusing on ER stress-related pathways.

Sigma-1 receptor (Sig1R) is associated with neuropsychiatric disorders such as delirium, schizophrenia, and PTSD[29]. Sig1R is abundant in MAM (mitochondrial-associated ER membrane) and colocalizes with GRP78/BiP and other proteins[30]. This receptor exerts antiapoptotic property in ER stress mitigation by acting as the inhibitor of proapoptotic proteins, and via the disassociation with GRP78 to enhance both proteins functions in mitigating the adverse effects of excessive or prolonged ER stress on cell survival[31, 32]. Under dormant state, Sig1R establishes intraluminal complex with GRP78. If an agonist binds to Sig1R, this complex will disassociate and ultimately will lead to the activation of Sig1R[29]. Inhibition of, or antagonism against Sig1R may stabilizes Sig1R-GRP78 complex and reduces chaperoning activities of these antiapoptotic proteins[31].

Selective Serotonin Reuptake Inhibitors, Memory, and PTSD

Serotonergic neurons have important roles in learning process and memory, but the exact physiology has not been elucidated[33]. Serotonin is known to promote learning ability and memory[34]. Hence, the administration of positive modulator of serotonergic neurons function, such as selective serotonin reuptake inhibitors (SSRI) antidepressant is expected to exert beneficial effects on animal and human hippocampal function, especially memory regulation.

Interestingly, sertraline is known to exert adverse effects on animal brain. Sertraline, which belongs to SSRI group, is a Food and Drug Administration (FDA)-approved drugs for PTSD[4]. This drug is assumed to act also as an inhibitor of Sig1R[35, 36]. Repeated administration of phencyclidine (PCP) (10 mg/kg/day, 10 days) to mice is linked to lower densities of Sig1R protein in frontal cortex and hippocampus, and also cognitive deficit. This deficit can not be reversed by sertraline administration, but can be treated by fluvoxamine (known as a SSRI antidepressant and Sig1R agonist) administration[35]. Fluvoxamine had been shown to potentiate nerve-growth factor (NGF)-

induced neurite growth of PC 12 cells. When the same cell line is treated with sertraline, the neurite growth-promoting effect can not be demonstrated[37]. Both sertraline and fluvoxamine are known to have high affinity to Sig1R[35, 37]. Recent study finds that sertraline induces hippocampal CA1 region pyramidal neuron disintegrity in dose-dependent manner in SPS-induced rodent model of PTSD[5]. These findings further promote the assumption that sertraline is an antagonist of Sig1R.

FDA warnings on sertraline preparations also include the possibility of increased risk of suicidality in patients. The authors propose that sertraline-induced Sig1R (and also its co-chaperone, GRP78) functional modulation may serve as a mechanism of SSRI-induced increased risk of suicidality in PTSD patient. Further examinations should be done to clearly elucidate the relationships between SSRI treatment, hippocampal structure and functions, and also PTSD clinicopathology. Hippocampal ER stress pathways and biomarkers, and pharmacodynamics of excessive ER stress-mitigating agents, such as fluvoxamine, must be studied in animal model of PTSD and individuals suffering from PTSD. This kind of study is expected to promote the establishment of effective pharmacotherapy for PTSD and its comorbidities.

Summary

Post-traumatic stress disorder (PTSD) is an anxiety disorder that is induced by the exposure to dramatic and/or life-threatening stresses in susceptible individuals. PTSD is accompanied by functional daily impairments and increased risk of suicide. At present only few drugs, if any, are clinically used to treat this condition. The use of selective serotonin reuptake inhibitor (SSRIs), the first line FDA-approved drug for PTSD, may increase the risk of suicidal ideation and suicide in patients suffering form the disorder. This clinical finding prompts the establishment of study focusing on the elucidation of SSRI pharmacodynamics in PTSD model. Recent study show that hippocampal ER stress is involved in single-prolonged stress (SPS)-induced rodent model of PTSD. SSRIs, such as fluvoxamine or sertraline, may contribute to the mitigation or exacerbation of mental conditions in PTSD patient, respectively. Glucose-regulated protein 78 (GRP78) (ER chaperone and master regulator of ER stress) is known to be involved in hippocampal ER stress in PTSD model. Sigma-1 receptor (Sig1R) (regulator of GRP78 chaperoning activity), is found to be activated and inhibited by fluvoxamine and sertraline, respectively. However, the exact roles of GRP78 and Sig1R in the hippocampus of animal model of PTSD have not been elucidated. Nevertheless, hippocampal ER stress remains interesting and novel candidate target for PTSD pharmacotherapy.

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REFERENCES

1. Wang HN, Peng Y, Tan QR, Chen YC, Zhang RG, Qiao YT, *et al*. Quetiapine Ameliorates Anxiety-Like Behavior and Cognitive Impairments in Stressed Rats: Implications for the Treatment of Posttraumatic Stress Disorder. *Physiol Res*. **59**:263-71 (2010).
2. Han F, Yan S, Shi Y. Single-Prolonged Stress Induces Endoplasmic Reticulum - Dependent Apoptosis in the Hippocampus in a Rat Model of Post-Traumatic Stress Disorder. *PLOS ONE*. **8**(7):1-12 (2013).
3. Yamamoto S, Morinobu S, Takei S, Fuchikami M, Matsuki A, Yamawaki S, *et al*. Single prolonged stress: toward an animal model of posttraumatic stress disorder. *Depress Anxiety*. **26**:1110-17 (2009).
4. Asnis GM, Kohn SR, Henderson M, Brown NL. SSRIs versus Non-SSRIs in Post-traumatic Stress Disorder An Update with Recommendations. *Drugs*. **64**(4):383-404 (2004).
5. Mahendra AN, Pramartha INT, Darmayanti NLS, Dewi NWS. Pharmaco-ethological and Hippocampal CA1 region neurohistological study of Sertraline effects on Single-prolonged Stress-induced Rodent model of PTSD. *Bali Med J*. **3**(3):S7-11 (2017).
6. Battal D, Yalin S, Eker ED, Aktas A, Sahin NO, Cebo M, Berköz M. Possible role of selective serotonin reuptake inhibitor sertraline on oxidative stress responses. *Eur Rev Med Pharmacol Sci*. **18**(4):477-84. (2014).
7. Mahendra AN, Efek Sertralin terhadap Memori Spasial dan Ekspresi *Glucose-regulated Protein*

78 pada Hippocampus Tikus Jantan Galur Wistar yang Diinduksi Single-Prolonged Stress (The Effect of Sertraline on Spatial Memory and Hippocampal Glucose-regulated Protein 78 Expression in Single-prolonged Stress-induced Male Wistar Rats) [Thesis]. Yogyakarta: Gadjah Mada University (UGM); (2015).

8. Lewy TG, Grabowski JM, Bloom ME. BiP: Master Regulator of the Unfolded Protein Response and Crucial Factor in Flavivirus Biology. *Yale J Biol Med*. **90**(2):291-300. (2017).
9. Gifford JB, Huang W, Zeleniak AE, Hindoyan A, Wu H, Donahue TR, Hill R. Expression of GRP78, Master Regulator of the Unfolded Protein Response, Increases Chemoresistance in Pancreatic Ductal Adenocarcinoma. *Mol Cancer Ther*. **15**(5):1043-52. (2016).
10. Oslowski CM, Urano F, Measuring ER stress and the unfolded protein response using mammalian tissue culture system. *Methods Enzymol*. **490**: 71-92. (2011).
11. Chong WC, Shastri MD, Eri R. Endoplasmic Reticulum Stress and Oxidative Stress: A Vicious Nexus Implicated in Bowel Disease Pathophysiology. *Int J Mol Sci*. **18**(4):1-19. (2017).
12. Hayashi T. Conversion of psychological stress into cellular stress response: roles of the sigma-1 receptor in the process. *Psychiatry Clin Neurosci*. **69**(4):179-91. (2015).
13. Serafini G. Neuroplasticity and major depression, the role of modern antidepressant drugs. *World J Psychiatry*. **2**(3):49-57 (2012).
14. Wible CG. Hippocampal Physiology, Structure and Function and the Neuroscience of Schizophrenia: A Unified Account of Declarative Memory Deficits, Working Memory Deficits and Schizophrenic Symptoms. *Behav Sci*. **3**:298-315 (2013).
15. Berumen LC, Rodríguez A, Miledi R, García-Alcocer G. Serotonin Receptors in Hippocampus. *Sci World J*. **2012**:1-15 (2012).
16. Piai V, Anderson KL, Dewar C, Parvizi J, Dronkers NF, Knight RT. Direct brain recordings reveal hippocampal rhythm underpinnings of language processing. *PNAS*. 113(40):11366-71 (2016).
17. Joca SRL, Ferreira FR, Guimarães FS. Modulation of stress consequences by hippocampal monoaminergic, glutamatergic and nitrergic neurotransmitter systems. *Stress*. **10**(3):227-49 (2007).
18. Jurgens CW, Hammar HM, Lichter JA, Boese SJ, Nelson BW, Goldenstein BL, *et al*. Alpha2A adrenergic receptor activation inhibits epileptiform activity in the rat hippocampal CA3 region. *Mol Pharmacol*. **71**(6):1572-81 (2007).
19. Hossain MM, DiCicco-Bloom E, Richardson JR. Hippocampal ER Stress and Learning Deficits Following Repeated Pyrethroid Exposure. *Toxicol Sci*. **143**(1):220-28 (2015).
20. Astur RS, Germain SAS, Tolin D, Ford J, Russell D, Stevens M. Hippocampus Function Predicts Severity of Post-Traumatic Stress Disorder. *Cyberpsychol Behav*. **9**(2):234-40 (2006).
21. Baumeister P, Luo S, Skarnes WC, Sui G, Seto E, Shi Y, *et al*.. Endoplasmic Reticulum Stress Induction of the Grp78/BiP Promoter: Activating Mechanisms Mediated by YY1 and Its Interactive Chromatin Modifiers. *Mol Cell Biol*. **25**(11):4529–40 (2005).
22. Ishisaka M, Kudo T, Shimazawa M, Kakefuda K, Oyagi A, Hyakkoku K, *et al*. Restraint-Induced Expression of Endoplasmic Reticulum Stress-Related Genes in the Mouse Brain. *Pharmacol Pharm*. **2**:10-16 (2011).
23. Racek T, Buhlmann S, Rüst F, Knoll S, Alla V, Pützer BM. Transcriptional repression of the Prosurvival Endoplasmic Reticulum Chaperone GRP78/BIP by E2F1. *J Biol Chem*. **283**:34305-14 (2008).
24. Kogure K, Nakamura K, Ikeda S, Kitahara Y, Nishimura T, Iwamune M, *et al*. Glucose-Regulated Protein, 78-kilodalton as a Modulator of Luteinizing Hormone Receptor Expression in Luteinizing Granulosa Cells in Rats. *Biol Reprod*. **2012**:1-29 (2012).
25. Jager R, Bertrand MJ, Gorman AM, Vandenabeele, P, Samali A. The unfolded protein response at the crossroads of cellular life and death during endoplasmic reticulum stress. *Biol Cell*. **104**:259-70 (2012).
26. Logue SE, Gorman AM, Cleary P, Keogh N, Samali A, Currents Concepts in ER Stress-Induced Apoptosis. *J Carcinog Mutagen*. **6**:1-7 (2013).
27. Fujita E, Kouroku Y, Jimbo A, Isoai A, Maruyama K, Momoi T. Caspase-12 processing and fragment translocation into nuclei of tunicamycin-treated cells. *Cell Death Differ*. **9**:1108-14 (2002).
28. Shiraishi H, Okamoto H, Yoshimura A, Yoshida H. ER stress-induced apoptosis and caspase-12 activation occurs downstream of mitochondrial apoptosis involving Apaf-1. *J Cell Sci*. **119**:3958-66 (2006).
29. Hashimoto K. Activation of sigma-1 receptor chaperone in the treatment of neuropsychiatric diseases and its clinical implication. *J Pharmacol Sci*. **127**:6-9 (2015).
30. Malhotra JD, Kaufman RJ. ER Stress and Its Functional Link to Mitochondria: Role in Cell Survival and Death. *Cold Spring Harb Perspect*

Biol. **3**:1-13 (2011).

31. Hayashi T, Su T. Sigma-1 Receptor Chaperones at the ER-Mitochondrion Interface Regulate Ca2+ Signaling and Cell Survival. *Cell*. **131**:596–610 (2007).
32. Pal A, Fontanilla D, Gopalakrishnan A, Chae Y, Markley JL, Ruoho AE. The sigma-1 receptor protects against cellular oxidative stress and activates antioxidant response elements. *Eur J Pharmacol*. **682**:1-19 (2012).
33. Monleon S, Vinader-Caerols C, Arenas MC, Parra A. Antidepressant drugs and memory: Insights from animal studies. *Eur Neuropsychopharmacol*. **18**:235-48 (2008).
34. Noori N, Bangash MY, Motaghinejad M, Hosseini P, Noudoost B. Kefir protective effects against nicotine cessation-induced anxiety and cognition impairments in rats. *Adv Biomed Res*. **3**:1-7 (2014).
35. Ishima T, Fujita Y, Kohno M, Kunitachi S, Horio M, Takatsu Y, *et al*. Improvement of Phencyclidine-Induced Cognitive Deficits in Mice by Subsequent Subchronic Administration of Fluvoxamine, but not Sertraline. *Open Clin Chem J*. **2**:7-11 (2009).
36. Hindmarch I, Hashimoto K, Cognition and depression: the effects of fluvoxamine, a sigma-1 receptor agonist, reconsidered. *Hum Psychopharmacol Clin Exp*. **25**:193–200 (2010).
37. Nishimura T, Ishima T, Iyo M, Hashimoto K. Potentiation of Nerve Growth Factor-induced Neurite Outgrowth by Fluvoxamine: Role of Sigma-1 Receptors, IP3 Receptors and Cellular Signaling Pathways. *PLOS ONE*. **3**(7):1-9 (2008).