PSD-95 Stroke IHC Grant Proposal

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Background

Post Synaptic Density Protein 95 or PSD-95 is a scaffolding protein highly concentrated in the postsynaptic density (PSD) of excitatory synapses. It plays crucial roles in synaptic organization and is essential for glutamatergic synaptic signaling (Cheng et al., 2006). PSD-95 is a core component of the PSD, a dynamic molecular network essential for synaptic strength regulation and neuronal circuitry refinement. PSD-95 is structurally organized in different protein domains, including three conserved PDZ domains, a Src homology 3 domain, and a guanylate kinase-like domain. These interactions regulate the content of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) at dendritic spines, essential for basal synaptic transmission and long-term potentiation. PSD-95 serves as a molecular anchor, tethering various neurotransmitter receptors, ion channels, and signaling molecules, thus facilitating the assembly of macromolecular complexes crucial for synaptic transmission and plasticity. However, the organization of PSD-95's molecular architecture to support its functional properties remains unclear.

Previous studies have illuminated various facets of PSD-95 biology and its implications in neuronal physiology and pathology. Chen et al. (2011) employed electron microscopy and tomography to unveil the structural organization of PSD-95 within the PSD, emphasizing its indispensable role in maintaining synaptic architecture and function. Moreover, Dore et al. (2021) demonstrated PSD-95's protective effect against beta-amyloid-induced synaptic dysfunction by finding that drug inhibitors targeting PSD-95 depalmitoylation reverses beta-amyloid-induced synaptic deficits, shedding light on its therapeutic potential in Alzheimer's disease. In addition to its implications to neurodegenerative diseases, PSD-95 has also been implicated in various psychiatric disorders such as schizophrenia and bipolar disorder, De Bartolomeis et al. (2023) found that disturbances in molecular signaling at the postsynaptic density (PSD) may be linked to schizophrenia.

Despite these advancements, gaps persist in our understanding of PSD-95 dynamics and its precise contributions to synaptic physiology and pathology. One such gap lies in deciphering the molecular mechanisms governing PSD-95 regulation under pathological conditions, particularly in neurological disorders characterized by synaptic dysfunction.

The current study seeks to address this gap by investigating the role of PSD-95 in excitotoxicity-induced neuronal injury, with a focus on its potential as a therapeutic target for stroke. A characteristic of ischemic stroke, excitotoxicity causes excessive glutamate release and subsequent excitatory neurotransmission, which accelerates the death of neurons. Strong data from Ayuso-Dolado et al. (2021) and Ugalde-Triviño & Díaz-Guerra (2021) points to PSD-95 as a potential target for reducing excitotoxic neuronal damage, opening up new treatment options for stroke.

Expanding on the findings above, the current study aims to study the potential neuroprotective affects of PSD-95 cleavage/inhibition in rat stroke models. We aim to provide a thorough understanding of the mechanisms underlying the complex relationship between PSD-95, excitotoxicity, and neuronal survival by combining in vitro and in vivo approaches with biochemical, pharmacological, and histological techniques. By studying the affects of PSD-95 inhibition on stroke models, we can then figure out key molecular targets and pathways to help develop targeted interventions that can protect neurons from excitotoxic damage and improve outcomes in stroke patients.

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Experimental Procedure:

1. Stroke induction: Rats will undergo surgical induction of stroke via middle cerebral artery occlusion (MCAO).

2. **PSD-95 Inhibitors:** Treatment groups will receive a single intravenous injection of PSD-95 inhibitors Tat-NR2B9c or Tat-NR2B9c (Wang et al., 2018) at appropriate concentrations via intravenous injection immediately after stroke induction. Control groups will receive vehicle only

3. **Immunohistochemistry (IHC) Staining:** Brain tissue sections will undergo IHC staining using specific primary and secondary antibodies to visualize PSD-95 levels.

The Primary Antibody is Rabbit anti-PSD-95 manufactured by Cell Signaling Technology, no. 3450S, $325/100 µL . The Secondary Antibody is the Goat anti-Rabbit IgG (H+L) secondary antibody,Alexa Fluor 488conjugate from Thermo Fisher Scientific; no. A-11008 , $150/100 µL

Controls: Brain regions with high levels of PSD-95; the Hippocampus, the cortex and the penumbra region because it is a critical area in stroke research(Baron & Marchal, 1999). For the positive control, both the primary antibody and the secondary antibodies will be administered. Since there are no brain regions with no levels of PSD-95, the same brain regions Hippocampus, cortex and the penumbra regions will be looked at for the negative control but only the secondary antibody will be administered with a blocking peptide control so as to prevent PSD-95 staining

Analysis: Stained tissue sections will be imaged using fluorescence microscopy.

PSD-95 levels and localization in brain tissue sections will be quantified and statistical analysis will be conducted using the software R to evaluate the effects of PSD-95 inhibitors on PSD-95 expression levels and neuronal survival post-stroke.

References

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