

NJCBIR Final Report

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1. Original aims of the project

Inflammation is an important consequence of the brain response to injury, trauma, stress, and disease. A key component of inflammation in the brain is the production of interleukin-1 (IL-1), which subsequently elicits production of additional cytokines and growth factors, such as nerve growth factor (NGF). NGF is synthesized as the precursor proNGF, and can either be secreted as such or cleaved to mature NGF. Mature NGF binds to the Trk receptor and can promote neuronal survival, while proNGF binds to the p75 neurotrophin receptor and can promote neuronal death. The aims of the project were:

1. **To investigate inflammatory signals that regulate p75^{NTR} expression in hippocampal neurons.** p75^{NTR} is induced in hippocampal neurons after damage and mediates neuronal cell death. We will determine whether specific inflammatory molecules, such as interleukin-1 β , directly regulate expression of this receptor, and investigate the mechanisms of regulation.
2. **To determine whether IL-1 β signaling is sufficient and necessary for the induction of p75^{NTR} and subsequent neuronal cell death in vivo.** IL-1 will be injected directly into the hippocampus to assess whether this is sufficient to induce p75^{NTR} in the absence of injury. We will also use mice lacking the IL-1 receptor to determine whether there is failure to induce p75^{NTR} under inflammatory conditions, and whether p75-mediated neuronal loss is attenuated.
3. **To determine which form of NGF protein is specifically produced in the brain under inflammatory conditions.** NGF mRNA is elevated in neurons and glia after different types of injury, but which form of the NGF protein is produced is not known.

The goal of these studies was to define the mechanisms by which the p75^{NTR} is upregulated in hippocampal neurons after injury, and to investigate the regulation of proneurotrophin production that elicits neuronal loss via p75^{NTR}.

2. Project successes

We have succeeded in performing the studies related to all three specific aims as follows:

Aim 1, We demonstrated that the inflammatory cytokines IL-1 β and TNF α induced expression of p75^{NTR} in both hippocampal neurons and astrocytes in vitro. We further analyzed the intracellular signaling pathways that these cytokines activate in the different cell types, and demonstrated that the signaling mechanisms are both cytokine and cell type specific. This work was published, as indicated by #1 below.

In the course of the studies on cytokine signaling, we demonstrated that hippocampal neurons have a specific IL-1 signaling protein that is lacking in other cell types, and that specifies neuron-specific responses to the cytokine. This work was initiated as part of the NJCBIR project, and was leveraged for additional NIH funding as an R21, therefore both agencies are acknowledged for funding support. (#2 below).

1. Choi, S. and W.J. Friedman, Inflammatory cytokines IL-1 β and TNF α regulate p75^{NTR} expression in CNS neurons and astrocytes by distinct cell type specific signaling mechanisms, *ASN Neuro*, 1 (2), e00010, doi:10.1042/AN20090009, 2009

2. Huang, Y., D. E. Smith, O. Ibáñez-Sandoval, J. E. Sims, and W. J. Friedman, Neuron-Specific Effects of Interleukin-1 β are Mediated by a Novel Isoform of the IL-1 Receptor Accessory Protein, *J. Neurosci.* 31(49):18048 –18059, 2011

Aim 2, IL-1 injected directed into the brain is sufficient to induce p75^{NTR} expression in hippocampal neurons, however this induction alone is not sufficient to cause neuronal loss. When cultured hippocampal neurons are pretreated with IL-1, we demonstrated that the cytokine increases surface expression of p75^{NTR} and its co-receptor sortilin, making the neurons more vulnerable to subsequent challenge with proNGF. These results suggest a mechanism by which IL-1 exacerbates neuronal loss following many types of injury, but does not directly induce neuronal death. These studies, together with those for Aim 3 have been submitted for publication.

Aim 3, NGF mRNA is induced by inflammatory cytokines such as IL-1, and is also upregulated following many types of injury. The proNGF form of the protein is secreted following seizures and other types of injury. In these studies, we determined that infusion of IL-1 into the brain in the absence of injury, elicited increased secretion of mature NGF, not proNGF. Therefore, although IL-1 is sufficient to increase p75^{NTR} expression in hippocampal neurons, making the neurons more vulnerable, it does not increase proNGF secretion, and therefore by itself does not cause neuronal death.

3. Choi, S. and **W.J. Friedman**, Interleukin-1 β regulates p75^{NTR} expression in vivo and increases vulnerability to proNGF-mediated apoptosis, submitted

4. Implications

In these studies we have begun to understand some of the mechanisms that make neurons vulnerable to signals in their environment following injury. It has previously been established that IL-1 does not, by itself, cause neuronal death, but does exacerbate neuronal loss following many different types of injury. We have demonstrated that one of the mechanisms by which this cytokine acts is by induction of the p75^{NTR} and its re-localization of the neuronal cell surface. We have also previously demonstrated that this receptor plays a significant role in loss of neurons following seizure-induced injury. Therefore, preventing the inflammation associated with brain injury prevents many of the consequences that cause progressive neuronal loss, even after the initial injury. Our data suggest that the induction of p75^{NTR} is one of those consequences, and important information on the mechanisms by which inflammation enhances neuronal death after injury.

5. Continuation of the Research and other funding

The studies we performed demonstrated that IL-1 is an important signal regulating the induction of p75^{NTR} following injury, which exacerbates neuronal loss following brain injuries such as seizures and stroke. Our ongoing studies will now investigate the role of this death receptor in mediating neuronal loss in specific models of traumatic brain injury. For that purpose, we are applying for a new grant from the NJCBIR.

As indicated above, during the course of investigating IL-1 signaling, we initiated a related project to investigate mechanisms by which this cytokine signals in different cell

types, specifically neurons versus astrocytes. This related project was funded by an R21 NIH grant (NS076867 “Neuron-specific effects of IL-1 β).

6. Publications:

1. Choi, S. and W.J. Friedman, Inflammatory cytokines IL-1 β and TNF α regulate p75^{NTR} expression in CNS neurons and astrocytes by distinct cell type specific signaling mechanisms, *ASN Neuro*, 1 (2), e00010, doi:10.1042/AN20090009, 2009
2. Huang, Y., D. E. Smith, O. Ibáñez-Sandoval, J. E. Sims, and W. J. Friedman, Neuron-Specific Effects of Interleukin-1 β are Mediated by a Novel Isoform of the IL-1 Receptor Accessory Protein, *J. Neurosci.* 31(49):18048 –18059, 2011
3. Choi, S. and W.J. Friedman, Interleukin-1 β regulates p75^{NTR} expression in vivo and increases vulnerability to proNGF-mediated apoptosis, submitted
4. Friedman, W.J., Proneurotrophins, seizures, and neuronal apoptosis, *The Neuroscientist*, 16(3):244-52, 2010

7. Financial summary

Funds from this grant were used to support the salaries of a technician and a student, both of whom worked on aspects of this project. These funds also were used for animals, supplies and reagents for this project.