Title: Female specific RNA-binding protein, AUF1, increases peripheral hypersensitivity after repetitive ischemia with reperfusion injury

Mentor: Mike Jankowski and Meranda Quijas

CAPSULE:

Background: Myalgia, which is muscle pain, affects up to 33% of the worldwide population making this a prevalent cause of pain in our society, therefore the study of pain pathways is being observed.

Results: Females in the rodent model are negatively affected by p-AUF1 and have more severe and lengthy chronic pain like behavior.

Conclusion: The phosphorylation of AUF1 seems to increase chronic hypersensitivity in Females.

Significance: Learning about the sex dependent pain pathways is vital to understanding chronic pain management.

Introduction: The title of the research project is female specific RNA-binding protein, AUF1, increases peripheral hypersensitivity after repetitive ischemia with reperfusion injury. I completed research with Dr. Jankowski's lab within Cincinnati Children's hospital within the pain management/anesthesia division.

In the lab we focused on Myalgia, which is muscle pain, and it affects up to 33% of the worldwide population making this a prevalent cause of pain in our society. [1] We are currently studying the pathways causing acute hypersensitivity to develop into chronic hypersensitivity. Chronic hypersensitivity, in the clinical sense, is when patients have episodes of pain repeatedly and over a longer time. Women more often experience severe chronic pain from myalgia from data and they have a heightened sensitivity compared to men. [1] Disease based pain is experienced more often in females, one example being fibromyalgia, and ischemia with reperfusion injury often occurs in this disease. From now on I will refer to the ischemia with reperfusion injury as IR injury.

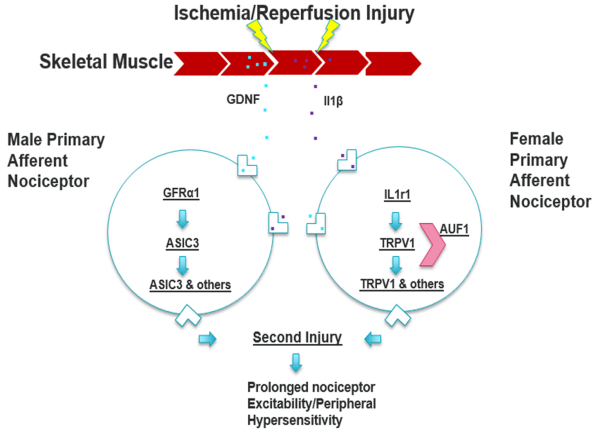

Figure 1

Materials and methods: This is why we used the IR injury in our research. We're looking at GDNF which is a muscle derived glial cell line-derived neurotrophic factor that signals to nociceptors and is upregulated in males post injury whereas AUF1, an AU-rich element RNA binding protein, is upregulated in female DRGs which we believe

interacts with IL1B upregulating IL1R1 post injury. DRG's are dorsal root ganglia - they are responsible for sensory signaling to the rest of the body. We believe phosphorylated AUF1 in the female pain pathway can lead to a heightened sense of pain-like behavior in comparison to males in the rodent model.[2] This comparison was made to observe if there is a distinct difference between the response to hypersensitivity between males and females.

In accordance with figure 1, GDNF upregulates ASIC3 which increases the GFRa1 receptors in the male which increases sensitization of group 3 and 4 afferents, leading to the development of chronic hypersensitivity but in females this chronic hypersensitivity development seems to be due to IL1B inducing TRPV1 which makes this mechanism sex specific. We've observed post IR injury an upregulation of GDNF in male DRGs and upregulated IL1B in the DRG of female mice- we believe the difference in these pathways being used is potentially due to enhanced expression of AUF1 in females, specifically phosphorylated or activated AUF1. A previous lab experiment showed a large concentration of phosphorylated auf1 in females in comparison to males post IR. [2] Therefore, to continue these experiments we performed a double IR injury with several treatment groups.

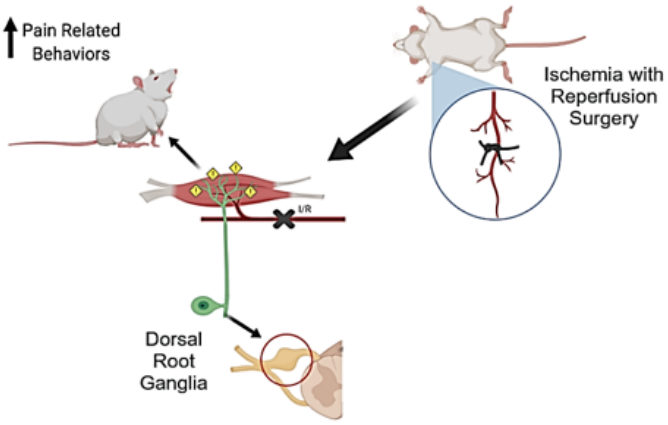

Figure 2

Ischemic myalgia presents differently in males and females, making chronic pain sex specific. Two IR's were done to make it into chronic hypersensitivity. The first IR makes the rodent experience acute hypersensitivity, then post the second IR it makes it into chronic pain-like behavior. The IR surgery is done by tying the right brachial artery to prevent blood flow for 6 hours then removing the knot for reperfusion injury as seen in figure 2. For our treatment groups, we used sham mice with no injury, Double IR mice with no injections, and two different nerve injected groups, on days 4 and 5 post single IR, mice were either injected with AUF1 (knocks down auf1), aav (injects single stranded dna genome to overexpress auf1in all cells in males) or a control and a PenCon group. [3]

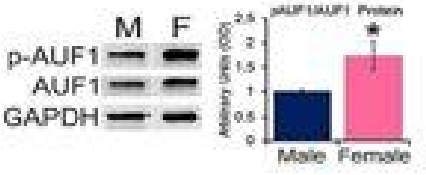

Figure 3

The behavior performed throughout the experiment included guarding and squeezing. For our molecular experimentation, one day post double IR we removed the DRG sections and some muscle samples from the right paw through dissection.

Results: We Started with inhibition of auf1 in males and females, affecting females not males. Suggested enhancement in female drg's. So that's why we did aav in males. [3]

Through the western blot (figure 3), it is shown that is not shown increases in females after double IR in comparison to males. However, in males GDNF increases significantly showing that there are sex differences in the mechanisms used post injury.

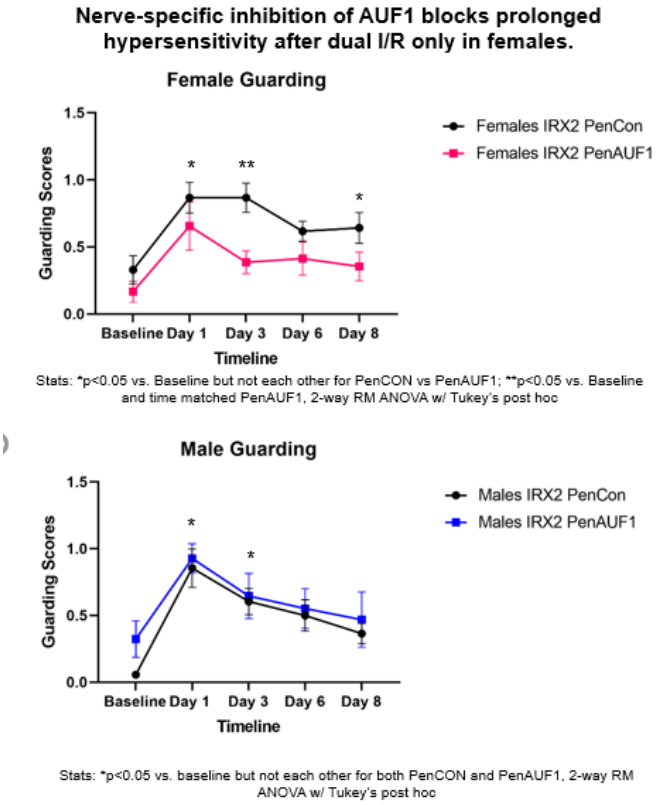

Figure 4

Pcr data shows compared to a naive mouse gfra1 is increased in males and females, increase in asic 3 in males in females it never increases. TRPV1 is increased in females not males.

Based on the data which is not shown, but was done previously, it can be concluded that the I/R injury does increase hypersensitivity. The injured mice had increased hypersensitivity in the right paw causing increased guarding when compared to sham mice.as you can see, AUF1 injected mice had decreased guarding compared to all of the treatment groups (figure 4).

Discussion: Overall, it was found that the phosphorylation of AUF1 can cause an increased hypersensitivity in females, knowing the p-AUF1 only occurs in the female pain pathway. However, in males, the phosphorylation of AUF1 does not occur and therefore lowers hypersensitivity. In treatment, blocking the activation of AUF1 may decrease intensity of female pain. In the future, we want to see if increasing AUF1 in males through an adeno-associated virus would cause males to phosphorylate auf1 and then use the IL1B pathway, increasing their hypersensitivity. Another direction is discovering what phosphorylates AUF1 causing the hypersensitivity in female mice and looking closely at that potential mechanism.

In a broader sense, our lab can compare old mice to a neonatal mice to see if there is an age related varying sensitivities in myalgia due to there being a potential sex related difference, and if our found results can be applied to all age groups..

The takeaway from this project was that pain happens to be sex specific and is likely based on the chosen pathway.

Acknowledgements: All of this could not be done without a team. I would love to thank Dr. Jankowski and Meranda Quijas for all of their help and support and the rest of the lab team for teaching me various techniques and skills. They were my biggest mentors and helped me grow in the lab this past semester.

This work was supported by grants from the NIH to MPJ (R01NS113965).

References:

E. Pierre Roy, Ludwig Gutmann [1] Myalgia, Neurologic Clinics, Volume 6, Issue 3, 1988, Pages 621-636, ISSN 0733-8619

Kumiko Tanabe, Kazumi Nishimura, Shuji Dohi, Osamu Kozawa, [2] Mechanisms of interleukin-1β-induced GDNF release from rat glioma cells, Brain Research, Volume 1274, 2009, Pages 11-20, ISSN 0006-8993

K. I. Berns, C. Giraud, [3] Adeno-Associated Virus (AAV) Vectors in Gene Therapy, 1996, Volume 218 ISBN : 978-3-642-80209-6