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

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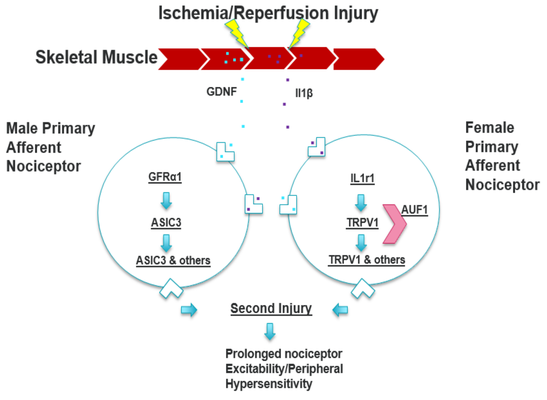
Introduction

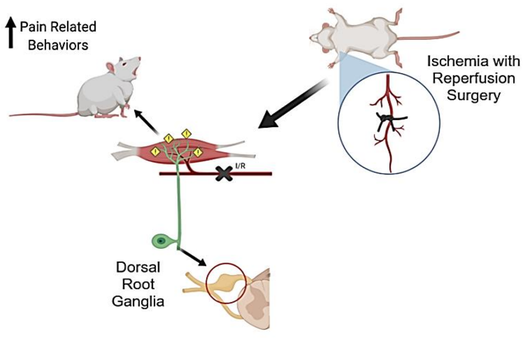
- Muscle pain is a frequent complaint of patients suffering from conditions associated with peripheral ischemia, such as peripheral artery disease or sickle cell anemia.
- Like many muscle pain conditions, patients with ischemic myalgia often report spontaneous/ongoing pain, mechanical hypersensitivity and muscle weakness.
- Females display higher prevalence and lower tolerance to myalgia.
- We have developed a model of ischemia with reperfusion injury (I/R), in which mice display these same behavioral and physiological alterations.
- Our previous preclinical studies suggest that peripheral mechanisms of myalgia development may be different between sexes.
- Both signaling from the affected muscle (GDNF and IL1β) and gene expression changes in the sensory neurons (ASIC3 and TRPV1) appear to be distinct in males and females with I/R injury.
- One gene that we have recently shown to be differentially upregulated in female dorsal root ganglia (DRG) is the RNA binding protein, AUF1, which has been linked to stabilizing distinct RNAs after transcription.
- We hypothesized that female specific expression of AUF1 regulated sex specific gene expression changes in DRGs that underly pain-like behaviors in mice with I/R.

Hypothesis



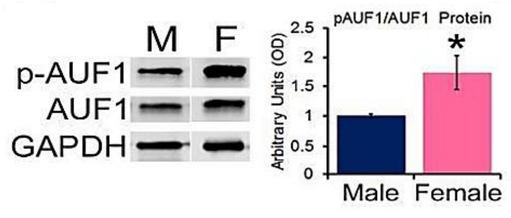
Methods

- **Animals:** All experiments were performed on young adult (21-60 days) Swiss Webster male mice.
- **Ischemia/reperfusion injury:** The right brachial artery was surgically occluded for 6h and allowed to re-perfuse for 18h as described in Ross et al (*J Pain*. 2014). Animals then underwent similar I/R injury 1 week later and animals analyzed as described.
- **siRNA injections:** Penetratin1 (Pen)-conjugated siRNAs (control or AUF1 targeting) were pressure injected into median and ulnar nerves 3d and 4d after the first I/R.
- **Adeno-associated virus (AAV) injections**: AAV serotype 9 expressing an AUF1 overexpression construct were injected into the median and ulnar nerve using similar methods as described for siRNAs 3 weeks prior to the first I/R in our groups.
- **Behavioral assessments:** Analysis of spontaneous pain-like behavior (forepaw guarding) was determined 1d before and 1d- 8d after second I/R was employed (Queme et al *J Neurosci.* 2016; Ross et al *J Neurosci.* 2016).
- **Gene expression:** SYBR Green real-time RT-PCR of whole DRGs or muscle was determined in mice with I/R injury at 24hr post second I/R and compared to naïve controls.
- **Western Blotting:** C7/C8/T1 DRGs were isolated, separated by PAGE and transferred to PVDF membranes prior to processing and imaging on a LiCor Odyssey imager. GAPDH normalized band intensity was quantified using Image-J.



Results

Phosphorylated AUF1 is significantly increased in female DRGs.



Stats: *p<0.05, 1-way ANOVA.

Results

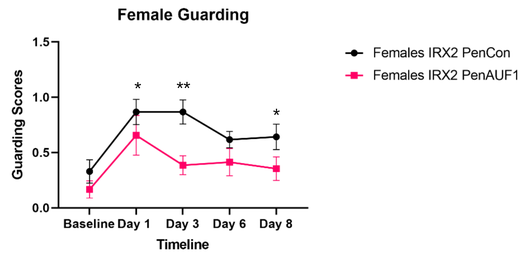
siRNA-mediated knockdown of AUF1 in sensory neurons inhibits select genes upregulated by dual I/R in females.

Male DRGs	2x I/R Alone	PenCON+ 2x I/R	PenAUF1+ 2x I/R
GFRα1	121±11%	183±29%	254±23%
ASIC3	204±34%	339±13%	676±6%
IL1r1	27±13%	54±22%	31±27%
TRPV1	-60±15%	-48±14%	-28±12%
AUF1	54±12%	47±8%	11±10%

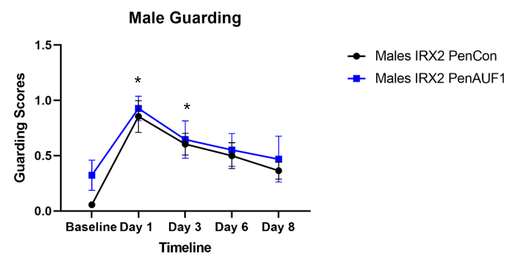
Female DRGs	2x I/R Alone	PenCON+ 2x I/R	PenAUF1+ 2x I/R
GFRα1	458±26%	541±26%	328±36%
ASIC3	-44±14%	69±13%^	24±21%
IL1r1	269±15%	231±16%	32±35%
TRPV1	62±19%	60±17%	9±27%
AUF1	137±27%	146±14%	31±31%

Stats: Yellow highlight = p<0.05 vs. naive, 1-way ANOVA w/ Tukey's post hoc

Nerve-specific inhibition of AUF1 blocks prolonged hypersensitivity after dual I/R only in females.

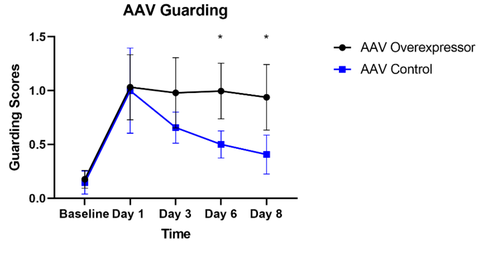


Stats: *p<0.05 vs. Baseline but not each other for PenCON vs PenAUF1; **p<0.05 vs. Baseline and time matched PenAUF1, 2-way RM ANOVA w/ Tukey's post hoc



Stats: *p<0.05 vs. baseline but not each other for both PenCON and PenAUF1, 2-way RM ANOVA w/ Tukey's post hoc

AAV-mediated overexpression of AUF1 in DRG neurons induces prolonged guarding behaviors after dual I/R in males.



Stats: *p<0.001 AAV OE vs AAV Control, 2-way RM ANOVA w/ Tukey's post hoc

Conclusions

- Dual I/R injury causes prolonged pain-like behaviors in both males and females.
- AUF1 is significantly higher in female DRGs compared to males DRGs at baseline.
- AUF1 targeting siRNAs injected female but not male mice caused inhibited I/R-related hypersensitivity.
- Results correlated to specific alterations in DRG gene expression in males vs. females with repeated I/R.
- Overexpression of AUF1 in males however was able to induce greater paw guarding after I/R compared to controls.
- Results could provide evidence for sex-specific treatment strategies for patients with ischemic myalgia.

Future Directions

- Determine whether there are male specific factors that regulate gene expression after dual I/R injury.
- Confirm the mechanisms by which AUF1 regulates sensory neuron gene expression after I/R.
- Assess whether results can be extrapolated to subjects of all age groups.

Acknowledgements

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