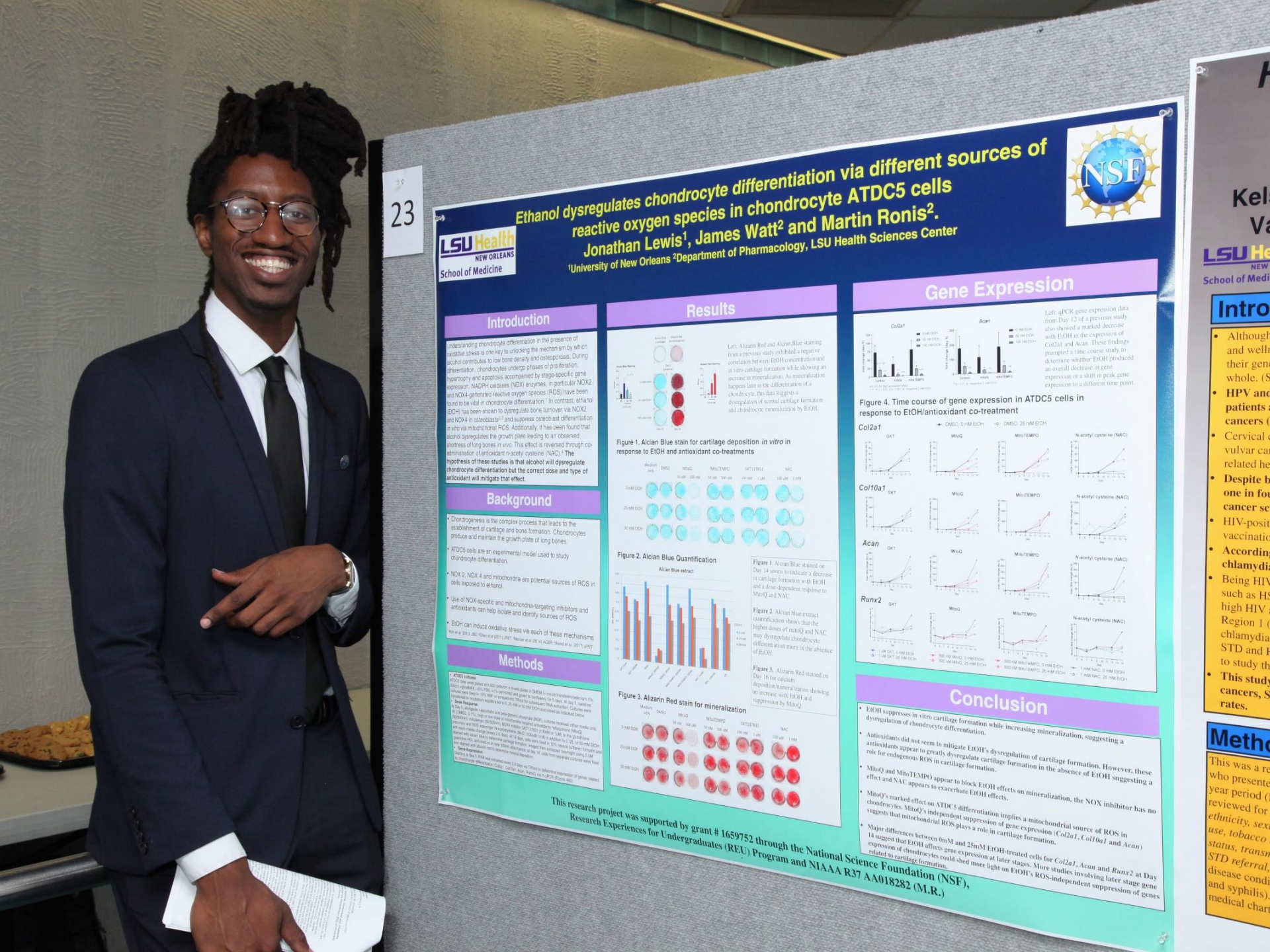
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LSU Health NEW ORLEANS School of Medicine
Ethanol dysregulates chondrocyte differentiation via different sources of reactive oxygen species in chondrocyte ATDC5 cells
Jonathan Lewis[1], James Watt[2] and Martin Ronis[2].
[1]University of New Orleans [2]Department of Pharmacology, LSU Health Sciences Center
NSF
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Background
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Figure 1. Alcian Blue stain for cartilage deposition in vitro in response to EtOH and antioxidant co-treatments
Figure 2. Alcian Blue Quantification
Figure 3. Alizarin Red stain for mineralization
Figure 4. Time course of gene expression in ATDC5 cells in response to EtOH/antioxidant co-treatment
This research project was supported by grant # 1659752 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program and NIAAA R37 AA018282 (M.R.)

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Purification and Functional Analysis of Monoclonal Antibodies Protection Against *C. auris* Invasive Infections

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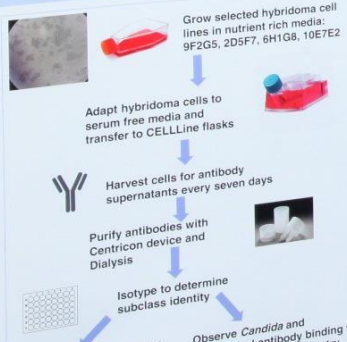
Introduction

Disseminated candidiasis is a life-threatening nosocomial infection, and is a leading cause of bloodstream infections affecting immunocompromised patients in the United States. Despite the advancements of antifungal therapies, the mortality rate remains unacceptably high. Due to its high mortality rate and burden on the healthcare system, novel approaches are needed to improve antifungal therapy. Among medically important *Candida* species, *C. auris* is a lethal fungus and has been identified over 700 cases in the United States as of July 2019. CDC refers to *C. auris* as a superbug because of its high mortality rate (30-60%), and it is multidrug resistant. This type of multidrug resistance has not been observed before in other species of *Candida*.

Preliminary data: Xin lab has identified and isolated a panel of monoclonal antibodies (mAbs) specific for *Candida* cell surface peptides, and the mAbs provide protection in mice against disseminated candidiasis caused by medically important *Candida* species, including *C. auris*.

Goals: The goal of this study was to produce protective mAbs by cell culturing hybridoma cells, adapt cells to CELLline flasks, and purify mAbs by using a Centricon device. Finally, ELISA, fluorescence staining and Flow cytometric analysis were performed to validate the binding of each mAb to specific *Candida* cell wall peptides.

Methods



Immunoassay Methods

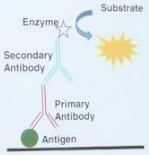


Fig. 1: ELISA was used to detect titers of purified mAb. EIA plates were coated with *Candida* cell wall peptides Fba, Met 6, GPV-P3, PgK1. Purified mAb were used as the primary antibody at varying dilutions. The secondary antibody was goat anti-mouse IgG-HRP. A similar method was used to determine mAb subclass identity.

Results

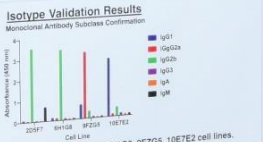


Fig 2: mAb subclass identity was verified for 2D5F7, 6H1G8, 9FZG5, 10E7E2 cell lines.

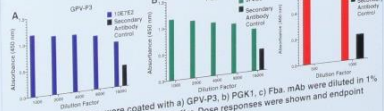


Figure 3: ELISA plates were coated with a) GPV-P3, b) PGK1, c) Fba. mAb were diluted in 1% bovine serum albumin (BSA) blocking buffer. Dose responses were shown and endpoint titers were recorded.

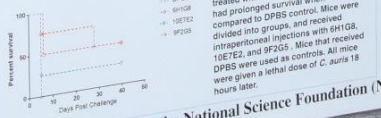


Fig 4: Preliminary data shows that mice treated with 6H1G8, 10E7E2, and 9F2G5 had prolonged survival when compared to DPBS control. Mice were divided into groups, and received intraperitoneal injections with 6H1G8, 10E7E2, and 9F2G5 . Mice that received DPBS were used as controls. All mice were given a lethal dose of *C. auris* 18 hours later.

Flow Cytometry and Fluorescent Staining

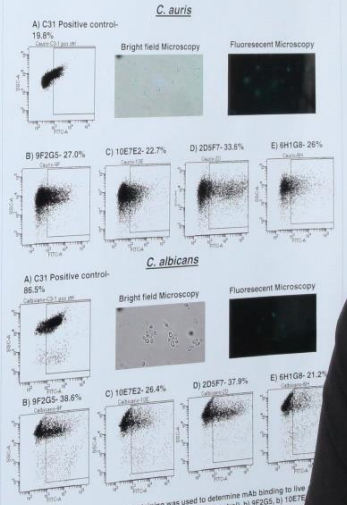


Fig 5: Flow cytometry and fluorescent staining was used to determine mAb binding to live *C. albicans* yeast cells. The figure shows a) C31 (positive control). b) 9F2G5. b) 10E7E2 6H1G8 mAb binding to *C. auris* (top panel) and *C. albicans* (bottom panel). Positive control observed under bright field and fluorescent microscopes.

Conclusions

- Immunoassay results show evidence of high functional titers for 10E7E2 and 9F2G5 which provide protection against disseminated infections against *C. auris* in mice.
- We observed antibody binding to live *Candida* cells through Flow cytometry.
- Monoclonal antibody based therapy may hold the hope for treating *C. auris* disseminated infections in humans.

This research project was supported by grant # 1659752 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Pro

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Retinal Sensitivity in Hormonally Modulated *Hyla cinerea* Using Electrophysiological Techniques

LSU Health NEW ORLEANS School of Medicine

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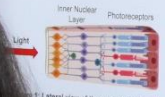
[1]Neuroscience Center of Excellence, [2]Department of Cell Biology and Anatomy, LSU Health, New Orleans, LA

Introduction

Hormones affect the performance of organs and tissues within the nervous system. The peripheral nervous system and its expanse of sensory receptors are the largest beneficiary from hormone modulation[1]. However, little is known of how endocrine mechanisms modulate visual signals and the performance of the retinal layer. Epidemiological studies have shown that there is a correlation between ocular diseases and a decline in sex steroids in the retina's sensory layer[2]. This project quantifies retinal function in the gravid, green tree frog *Hyla cinerea* to evaluate the effect of hormone modulation in visual systems. Reproductive states are hypothesized to increase retinal sensitivity, and have an effect on the performance of bipolar cells in the inner nuclear layer (INL) of the retina. Electroretinograms (ERGs) are the primary method for recording neural responses in the eye, and are used in this study to evaluate cellular activity under varying wavelengths and light intensities. Understanding the relationship between endocrine state and peripheral sensory function can potentially identify optimal biological conditions for retinal health.



1: Lateral view of the retina and its nuclear layers.

reproductive (endocrine) state affect Inner ar Layer Response to light?

y: Scotopic ERGs

males were dark adapted in a light proof

nistered to immobilize the frog during throughout the procedure.
eye to record the electrical response

es from isointensity light flashes at 50nm, 550nm, 600nm, 650nm, 700nm, ctral sensitivity.
amplitude across different light for individual wavelengths based on y Log(I)).

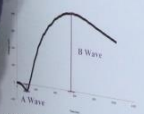
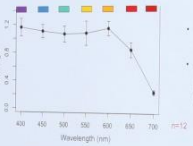


Figure 3: Graphical representation of an ERG response. The A wave represents the hyperpolarization of photoreceptors in the outer nuclear layer of the retina. The B wave is bipolar cell depolarization in the inner nuclear layer.

Results: Relative Spectral Sensitivity



- Bipolar cell activity at longer wavelengths is relatively less in comparison to shorter wavelengths.
- Analysis of relative spectral sensitivity provides a broad overview of visual limitations, without considering photoreceptor saturation.

Figure 4: Average B wave amplitude for all experimental groups during isointensity (2.157 cd/sm^2, 0.33 log cd/sm^2) stimulation at different wavelengths of light. F6,74=9.76; P<0.001.

Results: V Log(I) Response Curves

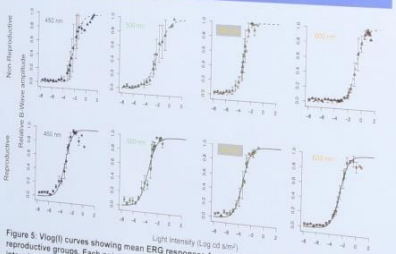
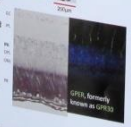


Figure 5: Vlog(I) curves showing mean ERG responses for female non-reproductive and reproductive groups. Each point represents the mean relative B wave amplitude at each light intensity. Reproductive threshold is shifted left.

Discussion and Future Experimentation on Endocrine Modulation

Assay precise endocrine state. A and B are non reproductive and reproductive ovaries, respectively.

Identify endocrine targets in photoreceptor and INL that could mediate modulation of estrogen receptor response. Image shows unstudied estrogen receptor in the INL.



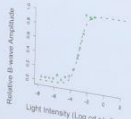


Figure 6: V Log(I) curve showing relative B wave amplitude at each light intensity for an hCG injected female. Compare to figure 5.

Discussion and Future Experimentation on Endocrine Modulation

- Relative spectral sensitivity data reveal the variation between non-reproductive and hormonally modulated retinal response.
- Retinal thresholds for individual wavelengths were calculated from ERGs following protocol 2. Hormonally modulated *Hyla cinerea* display a lower retinal threshold compared to non-reproductive groups.
- Bipolar cell response is emphasized in ERG traces. Further investigation into direct hormonal activity on Bipolar cells is needed to fully understand the underlying mechanisms mediating this effect.
- Clinical Significance: This modulation may enable a therapeutic tool to increase sensitivity in damaged retina

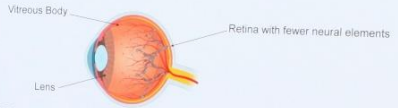


Figure 7: Eye with retinitis pigmentosa. Gray regions mark deterioration of the retina and vascular physiology. Individuals with this condition experience night blindness and progressive loss of the visual field.

Future Work

- Enzyme Linked Immunosorbent Assays will be used to verify hormone concentrations within the blood.
- Opsin sequencing will be conducted via Fluorescence in-situ hybridization to investigate location and advanced function in the retina.
- Computation of optimal retinal function and correlating reproductive hormone concentration has potential clinical applications for treating degenerative retinal diseases.

Acknowledgements

Preliminary data and images were collected with the assistance of doctoral candidate Whitney Walkowski and principal investigator Dr. Hamilton Farris. A large thank you to the National Science Foundation for funding the 2019 Research Experiences for Undergraduates Program at Louisiana State University Health Sciences Center.

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The Protein S LG1+2 Domain Contributes Significantly to Inhibition of Factor IX_a

LSU Health NEW ORLEANS School of Medicine

Amber Sylvain, Sabyasachi Chatterjee, PhD, and Rinku Majumder, PhD

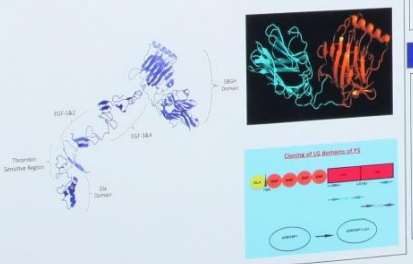
LSU Health Science Center.

Introduction

Protein S (PS) is a vitamin K-dependent anticoagulant. PS deficiency may have severe—even deadly—outcomes, such as deep vein thrombosis, arterial thrombosis (stroke, heart attack), and pulmonary embolism. We have demonstrated a direct inhibition of FIXa by PS independent of Protein C, in the absence and presence of FVIIIa. However, the binding sites for FIXa within PS have not been identified; such identification is a necessary first step to enlisting PS in the prevention of life-threatening thrombosis. Thus, we used an *in silico* docking approach with the three dimensional structure of Sex Hormone Binding (SHBG) domain(s) of PS that predicted the involvement of SHBG domain to mediate FIXa binding. The SHBG is composed of mainly two laminin (LG)-type domains, LG1 and LG2. Anisotropy of active site-labeled FIXa (DEGR-IXa) in the presence of phosphatidylserine/phosphatidylcholine vesicles and increasing concentrations of LG1+2 constructs revealed a binding K_d of 50 nM, which was comparable to the binding K_d of intact PS to FIXa (K_d ~ 40 nM). We used Circular dichroism spectroscopy (CD), isothermal titration calorimetry (ITC), as well as *ex-vivo* plasma assays to identify the structural and functional interactions between LG domains of PS and factor IXa. CD spectroscopy identified the conformational changes, and ITC identified the association constant (*Ka*), binding stoichiometry (n), and thermodynamic parameters (ΔG, ΔS and ΔH) when the LG domains bind to FIXa. Furthermore, we used the physiologically relevant Thrombin Generation Assay (TGA) to identify whether LG domains could reduce peak thrombin formed in PS-deficient plasma. Finally, we determined aPTT clotting times of PS deficient plasma in the presence LG1, LG2 and LG1+2 domains. Furthermore, we determined that the LG1+2 domain, and LG1or LG2 domain could mimic the effect of intact PS in inhibiting FIXa.

Objectives

Which of the two Laminin G like domains—if not both—are responsible for binding to and inhibiting coagulation FIXa?



Circular Dichroism (CD)

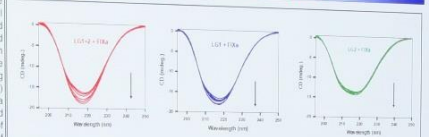


Figure 1. The change on CD spectra of LG1+2, LG1, and LG2 (2.5μM) domain of PS with binding with 0.0,25,0.5,0.75,1, and 1.25μM of FIX_a. The conformational changes of are 15.49%, 9.04%, and 3.02 % for LG1+2, LG1 and LG2 respectively.

Isothermal Titration Calorimetry (ITC)

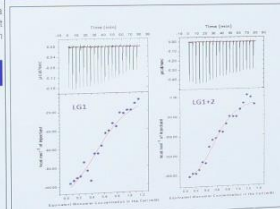


Figure 2. ITC of the interaction of LG1 and LG1+2 domains of PS with FIXa. The top panel shows raw data of sequential concentrations of FIXa into LG1 and LG1+2 doamins. The bottom panel represents the integrated heat result of the titrations.

ITC Data

	LG1	LG1+2
n	0.649	0.6
K_a	5.16 X 10^6 M^{-1}	1.2 X 10^7 M^{-1}
ΔG	-8.9 kcal/mol	-9.4 kcal/mol
ΔH	-73.25 kcal/mol	-239 kcal/mol
$T\Delta S$	-64.16 kcal/mol	-216.2 kcal/mol

Table 1. Parameters determined by ITC between LG1 and LG1+2 constructs of PS with FIXa.

ex-vivo Plasma Assays Data

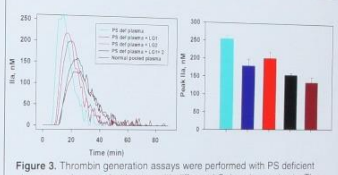


Figure 3. Thrombin generation assays were performed with PS deficient plasma supplemented without or with different LG domain constructs. The reactions were initiated with low TF (0.4 pm) to selectively generate thrombin in the intrinsic pathway. The thrombin generation was also performed with normal pooled plasma.

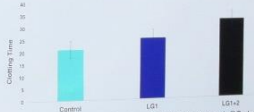


Figure 4. Clotting Assay showing the clotting times of PS defic plasma as a control and in the presence of LG1 and LG1+2 (S domains.

Conclusion

The CD spectra showed significant conformational changes however, the change in conformation was maximum with LG ITC data showed similar results, demonstrating that the intera and LG1+2 is exothermic, driven by a negative enthalpy concluded from the negative Gibbs free energy value(ΔG association constant (Ka) (Table 1), that the interaction be FIXa was comparatively more feasible than that of LG1 wi stoichiometry of bindings of both LG1+2 and LG1 with F binding of LG1+2 withy FIXa was more stronger than with clotting assay showed increased clotting time with the LG1+2, and TGA supported the results from the clotting a the addition of LG1+2 domain reduced the peak thrombin basis of these results, we conclude that the LG1+2 doma PS in binding to and inhibit FIXa.

This research project was supported by grant # 1659752 through the National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program

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Neuroadaptations in Animal Model of Comple Alcoholi

Amy P. Urbina, Jessica A. Cucinello, K

Department of Physiology, LSU Tular

Introduction

Behavioral A

Alterations in Estro

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actor IX$_a$
, PhD, and Rinku Majumder, PhD
ce Center.

ex-vivo Plasma Assays Data

Conclusion

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Neuroadaptations in Estrogen Signaling in an Animal Model of Complex Regional Pain Syndrome & Alcoholic Neuropathy

Amy P. Urbina, Jessica A. Cucinello, Kimberly N. Edwards, Liz Simon, Scott Edwards

Department of Physiology, LSU Health Sciences Center- New Orleans

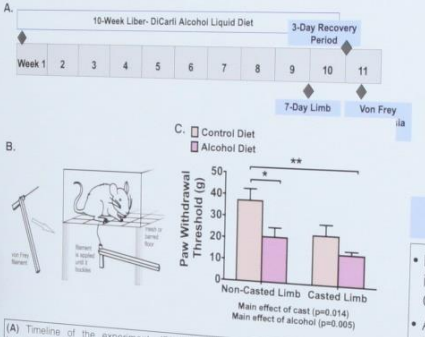
Tulane University

LSU Health NEW ORLEANS School of Medicine

Introduction

Complex Regional Pain Syndrome (CRPS) is a [illegible] occurs following injury [illegible] women.

Behavioral Analysis

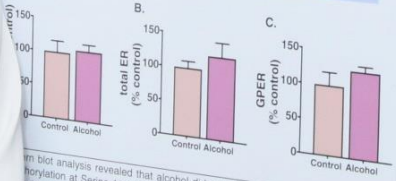


(A) Timeline of the experiment. (B) Visual Representation of the von Frey hyperalgesia test. (C) Additive effects of cast immobilization and chronic alcohol on hyperalgesia 3 days after cast removal using the Von Frey test (n=8-10 per group).

Alterations in Progesterone Signaling

(A) Western blot analysis revealed that alcohol increased progesterone receptor phosphorylation at Serine 294.

Alterations in Estrogen Signaling



Western blot analysis revealed that alcohol did not affect (A) estrogen receptor phosphorylation at Serine 118, (B) expression of total estrogen receptors, or (C) expression of GPER. (n=4-6 per group).

Conclusions

- Behavioral data demonstrate that chronic alcohol intake increased pain sensitivity in our model of CRPS.
- Although the difference in the expression of several estrogen receptors was not statistically significant, there was a general trend for alcohol to increase the expression of total ER and GPER.
- An alcohol liquid diet led to an increase in progesterone receptor phosphorylation at Serine 294 in the cingulate cortex.

Future Directions

- Future analysis will investigate other pain-related brain regions, including the prefrontal cortex and central amygdala.
- Because pain and motor information travel contralateral, future research will investigate if neuroadaptations to alcohol and CRPS are lateralized in the motor cortex in accordance with the injured limb.
- To determine associations between pain-like behavior and estrogen/progesterone signaling, we will analyze correlations between paw withdrawal threshold and protein expression.

...as supported by grant # 1659752 through the National Science Foundation (NSF), ...Research Experiences for Undergraduates (REU) Program