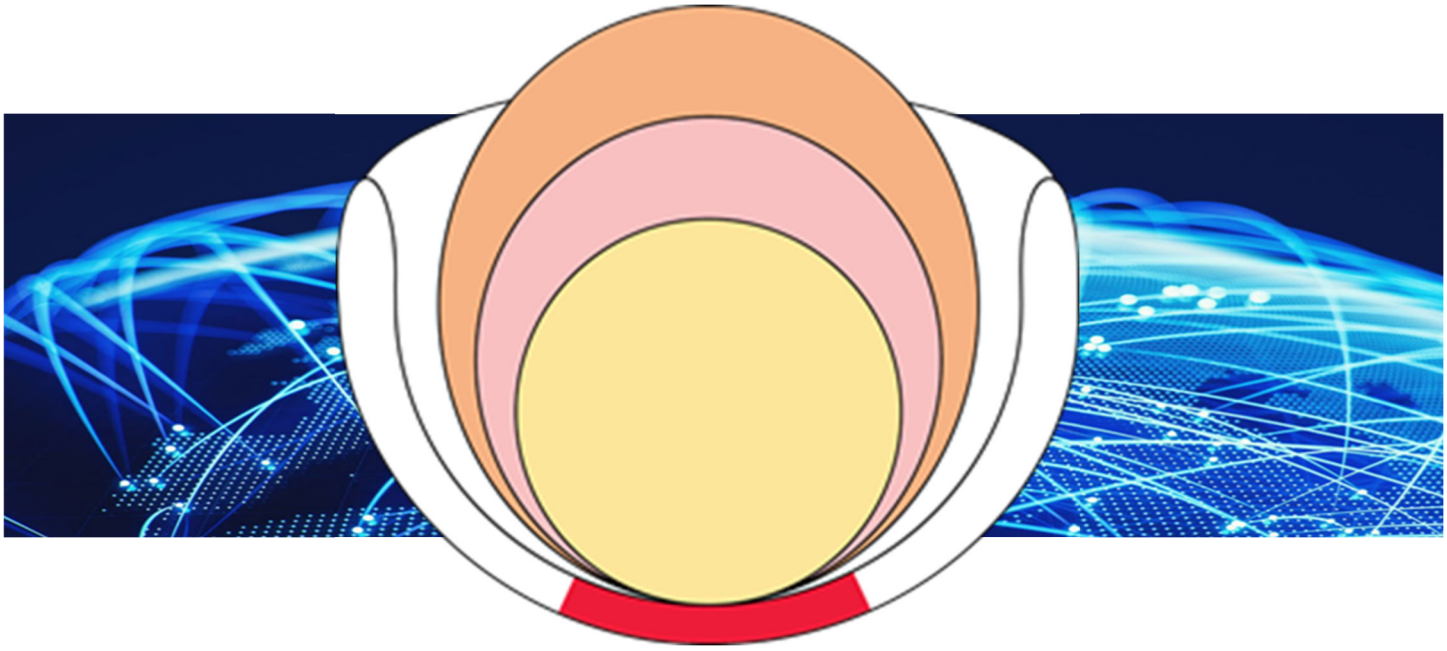
THE SOCIETY FOR PELVIC RESEARCH

SEVENTH ANNUAL MEETING



MEETING PROGRAM

December 8-10, 2022
Charlotte, NC

#PelvRes22

Sponsored in part by:

1R13 AG071166-01 from the
National Institute on Aging (NIA)

In Partnership with:

The International Continence Society

AUA Urology Research Conferences Advisory Board (URCAB)

The Sociedad Mexicana de Ciencias Urogenitales

Special Thanks to:

Ms. Elizabeth Foss CTC, MCC, MBA, President of My Travel Elf, Inc. / MTE Vacations, Naples, FL. Beth continues in her role for our in person meeting planning/facilitation.

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Meeting

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The 2022 SPR Trainee Awards Committee

Francis "Monty" Hughes, Jr., PhD
Mary F. Barbe, PhD

All those who attended and participated in the Seventh Annual Meeting

Our Mission Statement

To promote the highest standards of basic and translational science research directed toward understanding benign pelvic visceral and musculoskeletal function and dysfunction through education, interaction, and advocacy.

Our Vision Statement

The Society for Pelvic Research will be the premier professional organization for career basic and translational scientists and engineers interested in benign urogenital, distal gut and pelvic floor research.

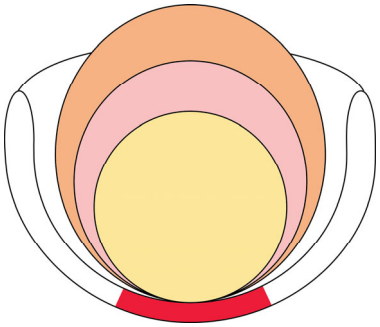
It will promote multidisciplinary interaction, intellectual cross-fertilization, networking for collaboration and career development through the regular dissemination of information via online resources, annual meetings and workshops, and published guidelines and standards for basic and translational science research.

Our History

The beginnings of the SPR trace back to the 2006 at a scientific meeting reception. Over refreshments, Matt Fraser and Mike DiSanto discussed starting a society that would primarily serve the needs of the career basic/translational researchers in the field of Pelvic Medicine. It took until December of 2013 to take that initial thought and do something about it. An email went out to the original group, and discussions and plans began. Additional Board Members were selected and invited to join in order to gain their expertise and a multidisciplinary balance.

After 7 years of Annual Meetings under three Presidents, we continue to forge new alliances with other scientific societies, have included a satellite symposium, and have been awarded an NIH R13 and have submitted an application for another.

Long Live the Society for Pelvic Research!



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Thursday, December 8, 2022

Trainee Affairs Committee Workshop

6:00 PM	**Moderators: Michael Odom, PhD and Matthew Fraser, PhD**	
	Emotional Intelligence: 5 skills to include in your Toolkit	**Britanny Knight, PhD**
	Career Exploration: Knowing your options and preparing for your next step	**Britanny Knight, PhD**
8:00 PM	**Trainee Social**	**Michael Odom, PhD**

Friday, December 9, 2022

8:00AM	**Breakfast**	
9:00 AM	**Welcome, Day 1 Main Meeting**	**Kelvin Davies, PhD**
	Session 1 Moderators: Molly Alexander and Kelvin Davies	
9:05 AM	**Keynote Address - From Bench to Bedside - a 30-year journey**	**Arnold Melman, MD**
9:55 AM	**Q & A**	
10:05 AM	Lumbosacral spinal proteomic changes during PAR4-induced persistent bladder pain: effect of spinal MIF antagonism.	Ye, Shaojing (Vera)
10:20 AM	Diabetic Bladder Dysfunction in Male Akita Mice Progresses Directly to an Underactive Phenotype in a NLRP3-Independent Manner	Hughes, Monty
10:35 AM	Detrusor contractility via FP receptor activation is increased in diabetic mice with bladder underactivity	Odom, Michael*
10:50 AM	Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox)-derived reactive oxygen species (ROS) modulate muscle function in dog bladders	Frara, Nagat
11:05 AM	Expression of nicotinic receptors in the intramural ganglion neurons of dog bladders	Frara, Nagat
11:20 AM	**Q & A**	
11:30 AM	**Lunch**	
	Session 2 Moderators: Anissa Cervantes and Margot Damaser	
12:30 PM	**State of the Art Speaker / ICS Ambassador - Pragmatic Regenerative Therapies for the Female Pelvic Soft Tissues**	**Marianna Alperin, MD MS**
1:20 PM	**Q & A**	**Q & A**
1:30 PM	Cremaster muscle denervation modifies some characteristics of the ejaculate	Varela Arzate, Rebeca
1:45 PM	Iodine supplementation reduces the expression and secretion of cytokines in the pathological prostate cell line stimulated with estrogens or tumor necrosis factor	Alvarez Cardenas, María de Lourdes
2:00 PM	An injection of botulinum toxin type A at the injured site of a spinal cord injury improves lower urinary tract function in female rats	Gandara, Cynthia*
2:15 PM	Non-genomic effect of estradiol on L6 dorsal roots response to glans penis stimulation in rats	Oloarte, Martín
2:30 PM	Transcutaneous electrical stimulation of the dorsal nerve of the clitoris differentially facilitates recovery of tonic and bursting activity of the striated urethral sphincter in	Juarez Mirto, Ricardo[+]
2:45 PM	**Q & A**	
2:55 PM	**BREAK**	

Friday, December 9, 2022

	Session 3 Moderators: Adam Doelman and Maryrose Sullivan	
3:10 PM	**Special Guest Speaker - Facilitating collaborative research in peripheral neuromodulation**	**Susan Tappan, PhD**
3:40 PM	**Q & A**	
3:50 PM	The Relationship of Anterior Vesical Branches and Pelvic Ganglia to the Ureter in Human Cadavers	Day, Emily
4:05 PM	Wireless Monitoring of Colonic Activity in Ambulatory Untethered Animals	Majerus, Steve (Damaser)
4:20 PM	Catheter-free Bladder Volume Monitoring Using Intravesical Sensor	Elazab, Mohamed*
4:35 PM	Optimizing Human Activity Classification to Improve Monitoring and Treatment of Lower Urinary Tract Dysfunction	Zareen, Farhath
4:50 PM	**Q & A**	
5:00 PM	**Close, Day 1 Main Meeting**	**Maryrose Sullivan, PhD**
5:30 PM	**Wine and Cheese Reception**	
7:00 PM	**President's Dinner for Faculty**	

Saturday, December 10, 2022

8:00AM	**Breakfast**	
9:00 AM	**Welcome, Day 2 Main Meeting**	**Maryrose Sullivan, PhD**
	Session 4 Moderators: Cara Hardy and Matthew Fraser	
9:05 AM	**State of the Art Speaker - Defining Phenotypes of Pelvic Organ Prolapse as First Steps Towards Personalized Medicine for Pelvic Floor Disorders**	**Kathleen Connell, MD**
9:55 AM	**Q & A**	
10:05 AM	Sensory Neuropeptides Modulate the ATP Hydrolysis by Soluble Nucleotidases in the Urinary Bladder Lamina Propria	Gutierrez-Cruz, Alejandro
10:20 AM	TRPM8 channels activation induces relaxation in pudendal arteries and corpus cavernosum from diabetic mice	Moraes, Raiana dos Anjos˚
10:35 AM	Urothelial Response to Short-term and Long-term Hypoxic Conditions	Hudson, Britney˚
10:50 AM	Characterization of cardiovascular function, metabolic status and erectile function in rat model of cardiometabolic syndrome	Pallas, Wrenn*˚
11:05 AM	Specialized pro-resolving mediator Resolvin E1 improves diabetic bladder dysfunction in male Akita mice	Cervantes, Anissa˚
11:20 AM	**Q & A**	
11:30 AM	**Lunch**	
	Session 5 Moderators: Soumya Kamath and Johanna Hannan	
12:30 PM	**Special Guest Young Investigator Speaker - Hydrogen sulfide protects erectile function through preservation of antioxidant defense**	**Justin La Favor, PhD**
1:20 PM	**Q & A**	**Q & A**
1:30 PM	Expression of an engineered chloride channel reduces bladder overactivity and inflammation in rats with cyclophosphamide-induced cystitis	Connelly, Jaclyn (Beckel)*
1:45 PM	Differential expression of myosin 5a isoforms in visceral organ innervation	Cristofaro, Vivian
2:00 PM	Targeted depletion of Fidgetin-like 2 (FL2) promotes cavernous nerve regeneration and improves erectile and bladder function outcomes in a rodent model of radical	Baker, Lisa (Davies)
2:15 PM	**Q & A**	

Saturday, December 10, 2022

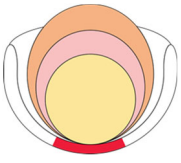
In Memoriam: Dr. Phillip P. Smith

2:25 PM	**The Phil Smith Legacy: Honoring a Life-Long Dedication to Advancing the Fields of Urinary Physiology and Bladder Aging**	**Cara Hardy, PhD**
2:55 PM	**Break**	
3:10 PM	**Trainee Awards Presentations**	**Mary Barbe, PhD and Monty Hughes, PhD**
3:50 PM	**Closing Remarks**	**Kelvin Davies, PhD**
4:00 PM	**MEETING ADJOURNS**	
6:30 PM	**Gala Dinner**	

*** - Top 5 Reviewed Abstract by the SPR Abstract Review Committee**
° - SPR Trainee Travel Awardees
+ - SPR/SMCU Travel Award

Top 5 Pre-Meeting Reviewed Abstracts (Abstract Review Committee)

Jaclyn Connelly	Expression of an engineered chloride channel reduces bladder overactivity and inflammation in rats with cyclophosphamide-induced cystitis
Mohamed Elazab	Catheter-free Bladder Volume Monitoring Using Intravesical Sensor
Wrenn Pallas	Characterization of cardiovascular function, metabolic status and erectile function in rat model of cardiometabolic syndrome
Michael Odom	Detrusor contractility via FP receptor activation is increased in diabetic mice with bladder underactivity
Cynthia Gandara	An injection of botulinum toxin type A at the injured site of a spinal cord injury improves lower urinary tract function in female rats



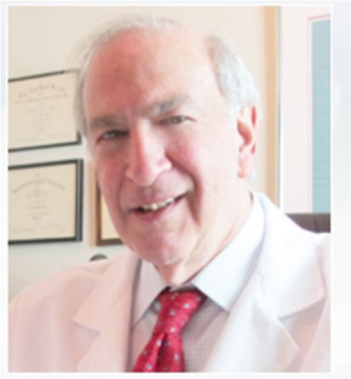
Brittany Knight, PhD

Bio: Dr. Brittany Knight is a trained Biomedical Scientist with a Bachelor's of Science degree in Psychology from Lock Haven University. In 2019, Brittany received her PhD in Biomedical Science from the University of Connecticut under the supervision of Dr. Kyle Baumbauer. Her thesis research focused on identifying the role of wound healing proteins called tissue inhibitors of matrix metalloproteinases (TIMPs) in the generation of acute inflammatory pain. After, she went on to conduct postdoctoral research in Lakshmi Nair's lab at the Connecticut Convergence Institute studying injectable biomaterials and local anesthetics for managing musculoskeletal pain.

Since 2018 Dr. Knight has volunteered with Beyond the PhD, formally known as Academia-Industry Opportunities Group (AIOG). In this role Dr. Knight has been instrumental in developing programs and providing resources to help students learn about their career options both within academia and beyond. These resources include the Career Discovery Seminar Series, Campus Ambassador Program, News and Career Blog, and networking events. These experiences inspired Brittany to pursue a career where she could combine her passions for science, mentorship, and career development. In 2021, following her postdoc, Dr. Knight started working for the United States Association for the Study of Pain (USASP). Currently she is the Director of Operations at USASP and works closely with the USASP committees to provide seminars and workshops, organize symposia, promote patient advocacy, and support trainees in the field.

Summary of Presentation: Join Dr. Knight for a 2-part interactive workshop, "Emotional Intelligence: 5 skills to include in your Toolkit" followed by "Career Exploration: Knowing your options and preparing for your next step". The format of the workshop will include a presentation combined with breakout activities to promote attendee networking and to complete short exercises relevant to the topics being discussed throughout the workshop. The first part of this workshop will cover what it means to be emotionally intelligent (EI), how to increase your EI, and why EI is essential for the workforce today. As a group we will review some scenarios that showcase the importance of EI in the workplace. The second part of the workshop will discuss alternative careers for PhDs to consider and will provide some tools to help attendees prepare for their next career step. We will also cover the use of social media for professional use, mentoring networks, diversity in the workplace, and how to stand out during the job application and interview process. Breakout sessions during this second part will include individual and group exercises to help attendees think outside the box and identify their needs when considering and preparing for a new career path.

Arnold Melman, MD



Bio: Dr Melman received his BS disagree at CCNY, MD from the University of Rochester School of Medicine, Urology training at the ULA Medical Center. He was a clinical associate at the Institute of Aging in the NIH and a Nephrology fellow at the Cedars Sinai Medical Center. After stints at Indiana University and Beth Israel Medical Center Dr. Melman became Professor and Chairman of the Department of Urology of the Albert Einstein College of Medicine and the Montefiore Medical Center from1988 to December 2012. He is now Professor Emeritus. He founded the Institute for Smooth Muscle Biology at Einstein. Dr. Melman is the author of more than 210 peer-reviewed journal articles, 42 book chapters and 20 patents. Dr. Melman was the Program Director of an NIH-funded $5 million, 5-year Program Project Grant entitled, "Smooth Muscle, Differential Tissue Function & Diabetes." He is the President of the Society of Salk Scholars of the City University of New York and a past co-editor of the International Journal of Impotence Research. He was an advisor to the United States Food and Drug Administration and Federal trade Commission. He co-founded Ion Channel Innovations, LLC in 2000 and has directed four, phase I and II clinical trials dedicated to the treatment of two common smooth muscle diseases erectile dysfunction and overactive bladder with the use of plasmid-based gene therapy with the Maxi-K gene.

Summary of Presentation: Our Urology laboratory research began in the mid 80's with the study by the study of the hormonal and neurotransmitter factors that influenced the relaxation and contraction of the smooth muscle of the penis. The work began at Beth Israel and then in1988 Moved to Albert Einstein. The laboratory was built from scratch in both facilities. Because urology is surgical department we had the advantage of being able to use human tissue obtained from well characterized patients that underwent first penile or bladder surgery in addition to animal models. In the early 90's we began the investigation of gap junction and ion channel control of smooth muscle tonicity of the penis and bladder function. Our discovery of the importance of potassium channel control of urologic organ function in health and disease led to the investigation of attempting to modulate that function with the use of plasmid-based gene therapy. Before preceding several patents written and approved. Because the negative effect of the Gelsinger viral- based gene therapy death in 1999 had placed multiple impediments in the path of using gene therapy to treat non-lethal, non-hereditary disease including interest by extant pharmaceutical companies. That created the need to form Ion Channel Innovations, LLC. to raise the necessary funding from interested patients and began the process of interacting with the CBER (Center for Biologics Evaluation and Research) the FDA group that controls gene therapy research. Two IND applications and RAC presentations were submitted for permission to begin. The National Institute of Aging, recognizing the need for new therapies to treat overactive bladder, supported one of the four clinical trials that were initiated. Results of the trials have been promising and without any significant therapy- related events and continue to advance.

Marianna Alperin, MD

Bio: Dr. Marianna Alperin obtained a Medical Degree with Distinction in Community Service at the St. Louis University School of Medicine in 2001. She went on to complete an Obstetrics and Gynecology residency at Brigham and Women's & Massachusetts General Hospitals, Harvard School of Medicine in 2005, followed by a three-year Female Pelvic Medicine and Reconstructive Surgery (FPMRS) fellowship at Magee-Womens Hospital, where she cultivated her passion for discovery science, and obtained a Master of Science degree in Clinical Research Design at the University of Pittsburgh. Upon conclusion of her fellowship in 2008, Dr. Alperin relocated to Los Angeles, where she assumed the founding Chief of the Division of Urogynecology position in the department of Obstetrics and Gynecology at Kaiser Permanente, West LA. Dr. Alperin's desire to facilitate the highest quality patient care through mechanistic research and the cultivation of interactions between basic, translational, and clinical scientists and health care providers from various disciplines led her to apply for a NIH Women's Reproductive Health Research (WRHR) position at the University of California, San Diego (UCSD). She joined the Department of Obstetrics, Gynecology, and Reproductive Sciences as an Assistant Professor and a WRHR Scholar in 2012. Currently, Dr. Alperin is a Professor and Fellowship Research Director at UCSD.

The mission of the Alperin lab is to discover scientifically rational treatment and prevention strategies for women's health, with special focus on pelvic floor disorders. Dr. Alperin's NIH-funded laboratory https://alperinmlab.org/ uses a multi- pronged approach, including computational modeling, in vitro and in vivo experimental models, cadaveric tissues, and biospecimens from living women, to study the impact of pregnancy, injury, radiation, obesity, menopause, and aging on the structure and function of pelvic soft tissues.

Summary of Presentation: Maternal childbirth injury is the leading risk factor for pelvic floor muscle dysfunction and the resultant pelvic floor disorders, which include pelvic organ prolapse and urinary and fecal incontinence. Despite high prevalence, significant morbidities, and economic burden associated with pelvic floor disorders, preventative strategies are almost non-existent, and the available treatments are delayed and compensatory as they do not directly target the underlying pathophysiology. To change the current clinical paradigm, Dr. Alperin has developed a close collaboration with Karen Christman, PhD, a Professor of Bioengineering at the UCSD Jacobs School of Engineering, with expertise in acellular injectable biomaterials. The long-term goal of the investigative team is the development of new, minimally invasive tissue-engineered therapies for the prevention and treatment of pelvic muscle dysfunction. Their studies of pelvic floor muscle morphometric properties in parous women with pelvic organ prolapse and the rat model of simulated birth injury demonstrate substantial degeneration, specifically cell death, myofiber atrophy and fibrosis. The above alterations render muscles insensitive to rehabilitation and are associated with poor clinical outcomes. As part of the multidisciplinary research program, the investigators used tissue-specific injectable extracellular matrix hydrogel, derived from decellularized porcine skeletal muscles, to promote endogenous regeneration of the pelvic skeletal muscles and to prevent pelvic floor muscle dysfunction post birth injury in a preclinical rat model, explicitly validated for the studies of the human pelvic floor muscles. The untreated and treated pelvic floor muscle phenotypic, functional, and transcriptional signatures were compared at multiple time points following birth injury. Collectively, these innovative studies provide fundamental knowledge of the biological processes involved in the pelvic floor muscle regeneration following maternal birth injury and a comprehensive assessment of the role of a low-cost acellular minimally invasive therapy in pelvic floor muscle recovery.

Kathleen Connell, MD

Bio: Dr. Kathleen Connell received her medical degree and completed her residency in Obstetrics and Gynecology from the State University of New York Downstate Medical Center. She then completed a fellowship in Female Pelvic Medicine & Reconstructive Surgery (FPMRS) at the Albert Einstein College of Medicine/Montefiore Medical Center. She is double board certified in Obstetrics and Gynecology and in FPMRS.

Dr. Connell is the Division Chief and William B. Goddard MD Endowed Professor in Urogynecology and Reconstructive Pelvic Surgery in the Department of Obstetrics and Gynecology at the University of Colorado School of Medicine. She is the Co-Director of the Women's Pelvic Health and Surgery, a multidisciplinary practice at the Anschutz Medical Campus. Her surgical practice focuses on treating pelvic floor disorders including pelvic organ prolapse, urinary and fecal incontinence, and reconstructive pelvic surgery.

Dr. Connell is a former scholar of the Yale School of Medicine Women's Reproductive Health Research Program (K12) sponsored by the Eunice Kennedy Shriver National Institute of Child Health and Human Development. Her translational research focuses on the effects of aging, menopause and other conditions on the pelvic connective tissues with the ultimate goal of preventing pelvic organ prolapse and developing new treatment options via personalized medicine. She is also a current fellow in the CU Innovations SPARK Program and developing a novel drug delivery system to treat recurrent urinary tract infections. She is actively involved in advancing scientific research in the field of urogynecology and serves on the Basic Science Subcommittee and Research Task Force for the American Urogynecologic Society.

Summary of Presentation: "Defining Phenotypes of Pelvic Organ Prolapse as First Steps Towards Personalized Medicine for Pelvic Floor Disorders"

Pelvic organ prolapse is a common, complex, and multifactorial condition whereby the pelvic support structures weaken, leading to descent of the pelvic organs and subsequent medical issues. Consistently demonstrated risk factors for primary prolapse include aging, parity, vaginal delivery and increasing BMI, while the only identified risk factor for recurrent prolapse after surgical repair is stage of prolapse at time of surgery. Unfortunately, there are no successful preventative measures to date, and surgical failure rates remain high. The current practice standards utilize the same treatment strategies for all patients.

In this talk, we will explore the hypothesis that weakening of pelvic support structures can occur via different mechanisms and may be the reason why the current uniform strategies for treating prolapse fail. Examples of genotyping and phenotyping pelvic support tissues to elucidate various mechanisms of pelvic support compromise may lead to more targeted individualized treatments for women with pelvic organ prolapse and improve long-term outcomes.

Susan Tappan, PhD

Bio: Susan Tappan is scientist with the Knowledge Core (K-Core) at the SPARC Data and Resource Center (DRC) where she is leading product management of SCKAN, a semantic store housing a comprehensive knowledge base of autonomic nervous system (ANS) nerve to end organ connectivity. She is particularly interested in improving the process by which connectivity information is derived from experts, open data, literature and textbooks.

Summary of Presentation: "Facilitating collaboration in peripheral neuroscience". The NIH Common Fund program, SPARC (Stimulating Peripheral Activity to Relieve Conditions), funds the basic, translational, and clinical research examining peripheral neuromodulation of organ function present on the SPARC portal. The SPARC Data and Resource Center (DRC) develops key technologies for the SPARC Portal so that contributed datasets are scientifically interpretable, FAIR (Findable, Accessible, Interoperable and Reusable), and compliant with the 2023 NIH Data Management and Sharing Policy.The DRC seeks to provide deep knowledge integration as a scientific and technological foundation for future bioelectronic medical devices and protocols. This presentation will describe the tools and goals of the SPARC DRC to deliver detailed, integrated, functional and anatomical maps and models of the peripheral nervous system and its innervation of target organs using FAIR data sharing principles.

Justin La Favor, PhD

Bio: Justin La Favor received his PhD in Bioenergetics from East Carolina University where his training and research focused on peripheral microvascular blood flow regulation in aging and obesity. He then completed a post-doctoral fellowship at Johns Hopkins University School of Medicine with Dr. Arthur Burnett in neuro-Urology and sexual dysfunction, where his research focused on redox regulation of penile hemodynamics. He opened his own laboratory in 2018 in the Department of Nutrition & Integrative Physiology at Florida State University where he is currently an Assistant Professor. His laboratory focusses on mechanisms that contribute to the progression of erectile dysfunction with emphasis on vascular physiology of the pelvic arteries and the corpus cavernosum. These studies primarily utilize knockout mice and animal models of diet-induced obesity, hypogonadism, and simulated spaceflight. His lab is currently funded by K01 and R03 grants from the NIDDK.

Summary of Presentation: "Hydrogen sulfide protects erectile function through preservation of antioxidant defense".

Obesity is a major risk factor for erectile dysfunction. Redox imbalance in the penis manifests early in response to an obesogenic stimulus. Hydrogen sulfide is a gaseous cell signaling molecule, the production of which has been shown to be diminished in various disease states resulting from obesity. Hydrogen sulfide has several anti-inflammatory and antioxidant properties that may protect against erectile dysfunction development. The potential impact of diminished penile hydrogen sulfide production on erectile dysfunction pathogenesis will be discussed, as well as possibilities of hydrogen sulfide as a therapeutic target for erectile dysfunction management.

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Title: Lumbosacral spinal proteomic changes during PAR4-induced persistent bladder pain: effect of spinal MIF antagonism.

Authors: Shaojing Ye[1], Nilesh M. Agalave[2], Fei Ma[1], Dlovan F. D Mahmood[1], Asma Al-Grety[2], Lin Leng[3], Camila I. Svensson[4], Richard Bucala[3], Kim Kultima[2], Pedro L Vera[1,5]

Affiliations: [1]Lexington VA Health Care System, KY, USA; [2]Dept of Medical Sciences, Uppsala University, Sweden; [3]Yale University, Yale, CT, USA; [4]Dept of Physiology and Pharmacology, Karolinska Institutet, Sweden; [5]Dept of Physiology, University of Kentucky, KY, USA.

Introduction/Objectives: Interstitial cystitis/Bladder pain syndrome is a painful condition with unknown etiology. Repeated PAR4 intravesical stimulation in mice results in bladder hyperalgesia (BHA) that lasts several days after the last stimulus with little or no evidence of bladder inflammation and is mediated by macrophage migration inhibitory factor (MIF) at the lumbosacral spinal cord. We aimed to describe spinal proteomic changes associated with persistent bladder pain and with spinal MIF antagonism.

Methods: Persistent bladder pain was induced in female (C57BL6) mice by three intravesical instillations (days 0, 2 and 4) of PAR4 agonist (100 uM; 1hr, scrambled peptide as control) under anesthesia. Lower abdominal hypersensitivity was measured by von Frey filaments on day 0-4, 7 and after intrathecal treatment: sham, anti-MIF monoclonal antibody (mAb) or isotype control. L6-S1 spinal segments were extracted on day 7, two hours after intrathecal (i.t.) injection and snap frozen and stored at -80°C. Total protein was extracted, reduced, alkylated and digested. After digestion, resultant peptides were labeled with a tandem mass tag 6 (TMT6) plex kit. TMT labeled samples were perfractionated using uHPLC system and analyzed by high-resolution nano LC-MS/MS using a Q-Exactive mass spectrometer. Data were analyzed using protein discover software and statistical analysis was performed using R studio. Pathway interaction was performed in the String analysis database.

Results: Repeated intravesical instillation of PAR4 agonist induced persistent bladder pain that was temporarily reversed (peak effect 2 hrs) by i.t. injection of anti-MIF mAb but not by isotype control or sham injection. Proteomics analysis identified and relatively quantified 6740 proteins. We identified 56 increasing proteins and 29 decreasing proteins in the persistent bladder pain group compared to scramble (no pain) control. Identified top increasing proteins were associated with nociception, inflammation, oxidative, mitochondrial, ribosomal and muscle-related factors while top decreasing proteins were associated with neuroprotection, redox modulation, mitochondrial factors, and neuronal related proteins. Intriguingly, neuroprotective proteins and mitochondrial-related proteins are protected by anti-MIF treatment. Pathway analysis of the top increasing and decreasing proteins shows significant pathway interactions in the string database.

Conclusion: Using unbiassed proteomic analysis we found that persistent bladder pain induced by PAR4 significantly altered the lumbosacral protein expression profile. Blocking MIF at the spinal level reversed persistent pain and prevented protein changes. Thus, central (spinal) protein changes are associated with persistent bladder pain and ongoing work will validate selected protein targets and examine their role in bladder pain.

Funding Source(s): DK121695; Swedish Research Council

Tilte: Diabetic Bladder Dysfunction in Male Akita Mice Progresses Directly to an Underactive Phenotype in a NLRP3-independent manner

Authors: *Francis M. Hughes, Jr.***,** Armand Allkanjari, J Todd Purves, Durham, NC

Affiliations: Department of Surgery, Division of urology, Duke University Medical School, Durham, NC

Introduction/Objectives: Diabetic Bladder Dysfunction (DBD) is a common complication of diabetes that may present with urinary frequency and urgency (i.e. overactive bladder (OAB)), detrusor underactivity and bladder decompensation (underactive bladder (UAB)), or some combination thereof. In an effort to define the etiology of this complication and develop models of its development, we have characterized the appearance of DBD in Akita Type 1 diabetic mice. Beginning with the female, which develop less severe diabetes, we demonstrated a transition from OAB at 15 weeks to UAB at 30. The purpose of this study is to explore the progression of DBD in the male mice of this diabetic model. We also explore a role for inflammation in this progression.

Methods: Four groups of mice (non-diab/NLRP3$^{+/+}$, non-diab/ NLRP3$^{-/-}$, diab/NLRP3$^{+/+}$, and diab/NLRP3$^{-/-}$) were evaluated at four different ages, 7, 10, 15 and 30 weeks. Awake, restrained cystometry was performed 7 to 9 days after suprapubic tubes (SPT) were implanted in the bladder. Blood glucose levels were measured prior to SPT placement. The extravasation of Evans blue dye assessed inflammation in the bladder. All parameters were assessed by ANOVA followed by Student-Newman-Keul's post-hoc using GraphPad InStat software (La Jolla, CA). Statistical significance was defined as $p<0.05$.

Results: Male mice at 7 weeks showed dramatically increased blood sugar at levels >500 mg/dL which were sustained through 30 weeks of age. In contrast, blood glucose in female diabetic mice are ≈280 mg/dL during this time. At 7 weeks we found there was no difference in void volume or urinary frequency between diabetic and non-diabetic mice (n=8,9). Beginning at 10 weeks the diabetic mice develop signs of UAB with a 55% decrease in urinary frequency and a 240% increase in void volume (n=9/11). At 15 (n=7/6) and 30 weeks (n=10/4) the decrease in frequency was similar (62% and 67%) as was the increase in void volume (305% and 312%). Surprisingly, unlike the females, deletion of the NLRP3 gene did not significantly affect any cystometric parameters. Using Evans blue we confirmed that inflammation was triggered in the bladder by diabetes and was blocked by deletion of NLRP3. In summary, DBD in male Akita mice presents initially as UAB and this pathophysiology does not change over time. While direct development of UAB is correlated with severity of hyperglycemia, NLRP3-dependent inflammation does not seem to play a dominant role.

Conclusions: Development of DBD in male Akita takes a significantly different trajectory from the female suggesting, besides NLRP3-mediated inflammation, alternative pathways can mediate the development of DBD.

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Detrusor contractility via FP receptor activation is increased in diabetic mice with bladder underactivity

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Introduction: Diabetic bladder dysfunction, affecting >50% of diabetics, is driven by bladder inflammation triggered by the NLRP3 inflammasome. Prostaglandin (PG) production in the bladder is dysregulated by inflammation but the inflammatory mechanisms responsible during diabetes, and how this disease affects PG-mediated bladder contractility, is unknown. Here, using the Akita genetic mouse model of Type 1 diabetes that develops bladder underactivity crossbred with NLRP3 null mice, we investigated how NLRP3-driven inflammation impacts PG release and bladder contractility.

Methods: Type 1 diabetic Akita mice were crossbred with NLRP3$^{-/-}$ mice to yield: control/NLRP3$^{+/+}$, diabetic/NLRP3$^{+/+}$, control/NLRP3$^{-/-}$, and diabetic/NLRP3$^{-/-}$ mice. Females were aged to 30 weeks when bladder underactivity is apparent. To measure *ex vivo* PG release, strips of isolated mucosa and detrusors were stretched to release PGE2 and PGF2α, which were quantified using ELISAs (n=4). To assess *ex vivo* smooth muscle contractility, contractile force of intact bladder strips in response to PGE2 and PGF2α +/- FP receptor antagonist AL8810, were measured.

Results: Diabetes doubled PGE2 released from the mucosa while reducing release from the detrusor by 67%. Surprisingly, no changes in PGE2 mediated bladder contractility were noted. In contrast, diabetes did not change PGF2α release in either the mucosa or the detrusor. However, intact bladder strips from diabetics demonstrated a 33% increase in PGF2α-mediated contractility which was abolished by AL8810. Diabetics lacking NLRP3, and therefore NLRP3-mediated inflammation, did not exhibit dysregulated PG release or PG-mediated bladder contractility.

Conclusions: Diabetes inversely affects PGE2 release from bladder mucosa and detrusor while not altering PGE2-induced contractions. Surprisingly, release of PGF2α was unaffected by diabetes although contractility to PGF2α, working through the FP receptor, was increased. This suggests that diabetes causes a change in FP receptor expression or signaling. These changes were driven by NLRP3-induced inflammation. The increased sensitivity to PGF2α suggests FP agonists may be effective therapies to treat existing underactive bladder dysfunction.

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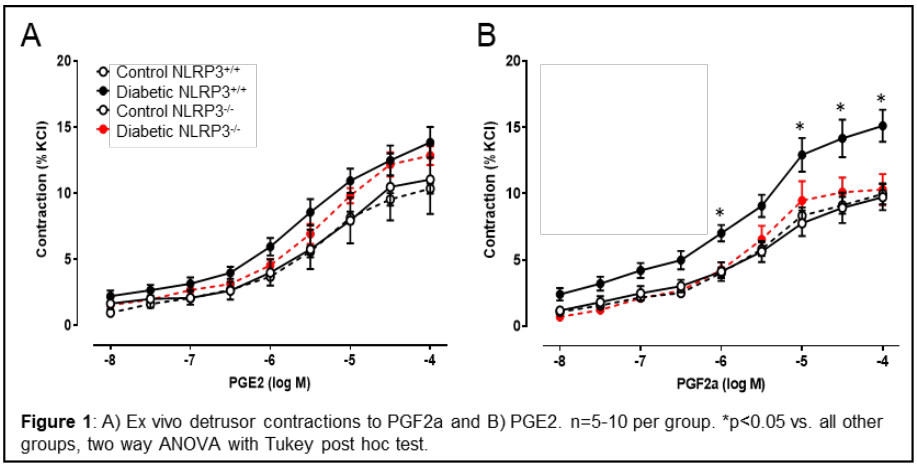

Figure 1: A) Ex vivo detrusor contractions to PGF2a and B) PGE2. n=5-10 per group. *p<0.05 vs. all other groups, two way ANOVA with Tukey post hoc test.

Title: Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox)-derived reactive oxygen species (ROS) modulate muscle function in dog bladders.

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Introduction and Objectives: Oxygen species are highly reactive metabolites of oxygen that can cause oxidative damage when excessively produced. Specifically, in urinary bladder tissues, reactive oxygen species (ROS) are mainly derived from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) enzymes. Although redox signaling processes receive much attention, their role in bladder pathology still needs to be investigated. Here, we utilized *in vitro* muscle strips contractility studies to further explore the physiological role of ROS/Nox and their involvement in modulating bladder function in normal dogs.

Methods: All studies were approved by the Institutional Animal Care and Use Committee according to guidelines of the NIH for the Care and Use of Laboratory Animals, USDA and AAALAC. Ten to eighteen mucosa-denuded bladder muscle strips obtained from four normal dogs (three males and one female) were mounted in muscle baths; then, on average, four strips were used from each animal, per treatment. Maximal contractile responses to 120 mM potassium chloride were measured. Trains of electrical field stimulation (EFS) of 1 ms pulses, 12 V, 8 Hz at 90 s intervals were applied to each strip for 20 min. Then, subsets of strips were incubated with ROS/Nox agonists or antagonists for 20 min in continued trains of EFS. About 15 min later, strips treated with antagonists were retreated with agonists and their responses examined. All responses are expressed in milli Newtons (mN).

Results: Application of exogenous ROS (hydrogen peroxide, H_2O_2; 100µM), enhanced EFS-induced smooth muscle strip contractions. Apocynin (100µM), the inhibitor of ROS generating enzyme Nox, attenuated intrinsic muscle strip activity. However, treatment with H_2O_2, restored EFS-induced muscle activity almost to control levels. Application of a key Nox activator and a pro-inflammatory peptide, angiotensin II (1µM), augmented EFS-induced smooth muscle contractions. Treatment with AT1 receptor specific antagonist, ZD7155 (10µM), inhibited EFS-induced activity, while retreatment with angiotensin II after ZD7155 blockade did not restore muscle activity.

Conclusions: The enhancement of EFS-evoked contractions by H_2O_2 and the inhibition of these contractions by the Nox inhibitor, apocynin, demonstrates a functional relevance of ROS in regulating smooth muscle activity and suggests that endogenous Nox-derived ROS regulates smooth muscle function. The augmentation of contractions by angiotensin II supports the involvement of Nox in bladder smooth muscle activity and confirms that the effects of angiotensin II is mediated by AT1 receptor. Collectively, these data provide evidence for a functional significance of Nox-derived ROS in dog bladders and that ROS can modulate bladder function without exogenous stimuli. Since inflammation is an important mechanism associated with oxidative damage, effects of angiotensin II on bladder smooth muscle function may have significant pathological implications.

Funding Source(s): NIA 1R01AG049321-01A1

Title: Expression of nicotinic receptors in the intramural ganglion neurons of dog bladders

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Introduction and Objective: Utilizing *in vitro* methods, we evaluated the expression of various neurotransmitter receptors mediating bladder function in control and long-term decentralized dogs.

Methods: All studies were approved by the Institutional Animal Care and Use Committee according to guidelines of the National Institute of Health for the Care and Use of Laboratory Animals and the United States Department of Agriculture and the Association for Assessment and Accreditation of Laboratory Animal Care. We used bladder tissues from 34 male and female dogs, divided into 3 groups: normal controls (9 females, 11 males), sham-operated controls (5 females), and decentralized (9 females). Decentralization involved bilateral transection of coccygeal and sacral spinal roots, and dorsal roots of lumbar 7, followed by 11-21 months post-operative recovery before euthanization and bladder tissue collection. Mucosa-denuded smooth muscle strips were mounted in muscle baths and maximal contractile responses to 120 mM potassium chloride (KCl) were measured. Strips were then incubated with neurotransmitter antagonists for 20 minutes, and contractions to epibatidine (nicotinic receptor agonist) determined.

Results: Male and female control bladders showed similar responses to KCl and epibatidine, as did control versus decentralized. Epibatidine induced contractions in control bladders were blocked by atropine (muscarinic receptor antagonist), atracurium and tubocurarine (neuromuscular nicotinic receptor antagonists), mecamylamine and hexamethonium, and SR16584 (antagonists to ganglionic and α3β4 neuronal nicotinic receptor subtypes, respectively), compared to vehicle treatment. Differentially, epibatidine responses in decentralized bladders were lower than control bladders after tetrodotoxin (a sodium channel blocker), and when exposed to lower concentrations of tubocurarine or SR16584. Also, decentralized bladders showed only partial blockade of epibatidine induced contractions after atracurium treatment.

Conclusions: In control bladders, since TTX did not block epibatidine contractions, nicotinic receptors are likely located on nerve terminals. In decentralized bladders, the antagonist pharmacology suggests a relocation of nicotinic receptors from nerve terminals to along axons distant from the neuromuscular junction (atropine and TTX blockade results), and changes in neuromuscular and neuronal (α3β4) nicotinic receptor profiles. Such changes after long-term extensive decentralization might suggest a physiological relevance for these nicotinic receptors in bladders as a compensatory mechanism to recover bladder function.

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Title: Cremaster muscle denervation modifies some characteristics of the ejaculate.

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Introduction/Objectives: In male rats, the cremaster muscle is a sac-shaped and is constituted by the convergence of the internal oblique and transverse abdominal muscles. Contraction and relaxation of the cremaster allow testicular descent and ascent, respectively, moving the testes away or closer to the abdominal cavity. Such movement results in the testicular temperature regulation 2-4°C lower than the body temperature. Thereby, the cremaster muscle contributes to the appropriate testicular thermoregulation maintaining the physiology of the testes i.e., steroidogenesis and spermatogenesis. The cremaster muscle is richly innervated by both branches of the genitofemoral nerve (Gfn), the femoral (lateral) and the genital one (medial). The Gfn can be damage due to inguinal hernias and sports hernias. Injuries to this nerve, probably change the contractile activity of the cremaster muscle thus the physiology of the testis.

The goal is to demonstrate the relationship between the inactivity of the cremaster muscle and the characteristics of the ejaculate at different days after cremaster muscle denervation.

Methods: Wistar male rats of 300 g of body weight were used. They were sexually trained during at least four copulatory tests. In anesthetized animals (ketamine 15 mg/kg and xylazine 1 mg/kg, IP) a midline abdominal incision was done to identify the descending aorta since parallel to it runs the Gfn. To avoid regeneration, a 1-cm portion of the nerve was dissected and cut bilaterally. Sham-operated male rats were submitted to the same procedure without cutting the nerve. At 20, 40 and 60 days post-neurectomy, the ejaculate of one ejaculatory series was obtained from the uterine horns of the mated female. After the seminal parameters and the seminal plug were analyzed

Results: GFn transection modified some seminal parameters but not the seminal plug (Table 1). Copulatory behavior did not show statistically significant changes. The postmortem examination of all the neurectomized animals revealed no evidence of regeneration.

Table 1. Altered parameters caused by Gfn in male-rats.

Seminal parameters	Sham (n=3)	20 DPN (n=4)	40 DPN (n=4)	60 DPN (n=4)
Progressive sperm motility (%)	77.6±1.7	76.5±0.91	61.25±4.01*	7.0±3.67**
Sperm viability (%)	91.33±5.01	93.25±1.67	89.25±1.45	9.6±0.35**
Sperm morphology (%)	99±0.1	98.75±0.35	99±0.1	99±0.1
Sperm count (10^6)	31.27±2.28	29.76±1.68	26.93±0.50	26.68±1.46
Seminal plug weight (mg)	80.33±5.30	82±6.95	97.25±12.45	91±9.94

Data are expressed as mean±ee. Statistical comparison was between sham vs 20 DPN or 40 DPN or 60 DPN.DPN=Days post-neurectomy.

Conclusions: Gf neurectomy causes progressive alteration in sperm motility from 40 DPN onwards and sperm viability from 60 DPN. Respect to sperm motility, it is known that it is acquired in the epididymis, but the sperm do not acquire it due to the permanent lack of contractility of the cremaster muscle. Since the sperm cycle lasts 54 days in the testis, and 10 days in the epididymis, the greatest loss of sperm motility is observed at 60 DPN. In addition to the functional epididymal alteration, there is a steroidogenic testicular failure, which results in a decrease in testosterone. This affects the accessory sexual glands, that are androgen dependent for what reduce the quantity and/or the quality of the substances that keep the sperm viable for fertilization.

Funding Source: CONACyT Fellowship 1150387 (RVA).

Iodine supplementation reduces the expression and secretion of cytokines in the prostate cancer cell line (DU145) stimulated with estrogens or tumor necrosis factor (TNF)

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Introduction: In the noncancerous prostate, deregulation in favor of estrogens (E_2) induces an inflammatory phenotype via ERα/ERβ, while in some types of cancer, E_2 induces anti-or proinflammatory actions. Molecular iodine (I_2) exerts antioxidant and antiproliferative actions in models of hyperplasia or prostate cancer, but its effects on inflammation are unknown. The objective of this study was to analyze the effects of I_2 on cell proliferation and interleukin 6 expression (IL6) in the absence or presence of E_2 or TNF in the ERβ+ positive DU145 cell line.

Methods: Dose-response analysis of E_2 (0.1, 1, 10, and 100 nM) and TNF (0.1, 1, 10, and 50 ng/ml) on cell proliferation were performed. Cells were treated with vehicle, 400 µM I_2 (IC50 previously reported), 10 nM E_2 and/or 10 ng/ml TNF. ERβ and IL6 gene expression was analyzed by real-time PCR, and secretion of IL6 into the culture medium by ELISA assay: Data were analyzed with one-way ANOVA and Tukey's post hoc test.

Results: 10 nM E_2 increased cell proliferation and I_2 reduced and prevented this increase in the E_2+I_2 group. Individual treatments with E_2 or I_2 decreased the expression and secretion of IL6, but these effects were not potentiated by the E_2+I_2 combination. E_2 and not I_2 decreased ERβ expression. On the other hand, TNF decreased proliferation in a dose-dependent manner and this antiproliferative effect was enhanced in the combined group (I_2 +TNF). As expected, TNF increased IL6 secretion, whereas I_2 alone and in combination with TNF suppressed the secretion of IL6.

Conclusions: The antiproliferative and anti-IL6 actions of I_2 prevailed in the presence of E_2. In presence of TNF, the antiproliferative actions of I_2 were enhanced and IL6 secretion was antagonized. Further studies are required to understand the relevance of the interaction I_2/estrogens/cytokines. The authors thank Dr. Evangelina Delgado for her technical support.

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Title: An injection of botulinum toxin type A at the injured site of a spinal cord injury improves lower urinary tract function in female rats.

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Introduction/Objectives: Decreasing urinary complications is a priority for patients with spinal cord injury (SCI). Currently there are no therapies to completely cure SCI and its complications, therefore it is necessary to evaluate new preclinical pharmacological approaches. Because botulinum toxin type-A (BoNT/A) can decrease the release of potentially neurotoxic neurotransmitters at the site of injury, we evaluated the effect of an acute, intramedullary injection of BoNT/A on lower urinary tract (LUT) function in female rats with a thoracic SCI by compression.

Methods: Wistar female rats had a compression SCI in the T8/T9 level (30g/5s), and immediately injected, intramedullary, with saline solution (5 microL) or BoNT/A (15 pg/5microL). Experimental groups were Sham+SS (N=4; laminectomy plus saline), Sham+BoNT/A (N=3; laminectomy plus BoNT/A injection), SCI+SS (N=4; SCI injected with saline), SCI+BoNT/A (N=4; injected with BoNT/A). Six weeks later we evaluated the function of the LUT by simultaneous cystometry and electromyography of the external urethral sphincter (EMG-EUS) in urethane-anesthetized rats. ANOVA was used to determine whether $p<0.05$.

Results: We found no differences in threshold pressure, void volume, maximum pressure or duration of high frequency oscillations between groups. The intercontractile range was significantly higher in SCI+SS rats (612.8±44.3s; $p<0.01$) when compared to the Sham+SS (239.3±57.6s), Sham+BoNT/A (300.6±90.8s) or SCI+BoNT/A (198.3±58.8s) groups. Rats from the Sham groups did not present bladder hyperactivity; however, the frequency of non-voiding contractions (NVC) was significantly higher in SCI+SS rats (1.13±0.07 NVC/min) than in SCI+BoNT/A (0.47±0.16 NVC/min; $p<0.01$). The EMG-EUS amplitude was 96.0±27.5µV in Sham+SS; 102.8±43.57µV in Sham+BoNT/A; 237.5±37µV in SCI+SS and 143.3±34µV in SCI+BoNT/A. The EUS-EMG duration was 59.4±6.6ms in Sham+SS; 51.57±6.4ms in Sham+BoNT/A; 291.3±79ms in SCI+SS and 124.0±19ms in SCI+BoNT/A. Although we found trends towards improved electromyographic parameters in SCI+BoNT/A rats, we found no statistical differences.

Conclusions: The intramedullary application of BoNT/A in SCI rats improved neurogenic LUT dysfunctions, particularly the intercontractile interval and bladder overactivity. Electromyographic parameters show a tendency towards normal values due to the application of BoNT/A. Further studies are necessary to confirm the usefulness of intramedullar BoNT/A injections in SCI rats.

Funding Source(s): PROSNI-2020-2021 Program, University of Guadalajara (to AM).

Title: Non-genomic effect of estradiol on L_6 dorsal roots response to glans penis stimulation in rats

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Introduction/Objectives: In male rats sensory activity of the glans penis is necessary for copulation. The sensory information travels from sensory receptors to the spinal cord through spinal dorsal roots, with L_1, S_1 and L_6 dorsal root ganglia as the main sensory suppliers. Mating behavior depends on gonadal hormones and has been described that estradiol is an important hormone for the recovery of mating behavior in gonadectomized male rats. However, it is unknown the physiological effect of estradiol on the sensory system of the glans penis. The aim of the present work was to determine the non-genomic effect of estradiol on the pattern of response of L^6 spinal dorsal root electrical activity during glans penis stimulation.

Methods: Wistar male rats between 270g-350g body weight were used. The rats were anesthetized with urethane to make it a laminectomy and discover the spinal dorsal root L_6. The root was cut near of the spinal cord. Then the spinal dorsal root proximal to dorsal ganglion was mount in stainless steel electrodes connected to an amplifier and this to an oscilloscope. Glans penis stimulation consisted in gentle pressure applied with a dissecting forceps. Dorsal root recordings were obtained before, one hour post-injection of estradiol receptors blocker and after estradiol benzoate replacement. Afterward, the animal was sacrificed with an overdose of pentobarbital.

Results: In an intact condition, gentle pressure of the glans penis produced phasic activity and tonic on and off responses. The estradiol receptors blocker did not significantly changed the pattern of the activity, although the tonic off activity was prolonged. Administration of estradiol benzoate reestablished the pattern of tonic off activity to the intact male rat levels.

Conclusions: Our results suggest that estradiol has mild non-genomic effect, via membrane receptors, on the activity of L_6 spinal dorsal roots discharged in response to glans penis stimulation in intact rats.

Funding Sources: Martín D. Oloarte received a scholarship from CONACYT (774989).

Transcutaneous electrical stimulation of the dorsal nerve of the clitoris differentially facilitates recovery of tonic and bursting activity of the striated urethral sphincter in rat model of childbirth trauma and motor nerve axotomy

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Introduction/Objectives: The EUS and its innervation is injured during childbirth which produces urinary incontinence. In a rat model of childbirth trauma, transcutaneous electrical stimulation of the dorsal clitoral nerve (TES) facilitates recovery of urinary continence. The objective of the present study was to determine whether TES-DNC improves EUS function in two models of urethra neuromuscular lesion, rats with EUS and nerve damaged by vaginal distension (VD,) and rats with EUS innervation injured by crush of the motor branch of the pudendal nerve (MBPdx).

Methods: Wistar strain rats were assigned to the groups (n=6 per group): Sham VD (SH-VD), VD+SH-TES, VD+TES, MBPdx+SH-TES and MBPdx+TES. VD (4 h) or MBPdx (crushing the nerve for 30 seconds). Immediately after the lesion and on days 2 and 3 post-injury TES was applied for 1 h (8.5 mA, 20 Hz). Electromyographic activity (EMG) of the EUS was evaluated during induced micturition in anesthetized animals (urethane, 1.2g/Kg, ip), at 7 days post-VD or 9 days post-nerve crush or sham surgery. One second of the EMG recorded before bladder contraction and during voiding were analyzed. Amplitude and frequency of the EMG signal were analyzed with ANOVA.

Results: In SH-VD animals the EUS discharged tonic and phasic activity, the EMG amplitude was greater ($p<0.001$) than that obtained in the other groups. At day 7 post-VD, tonic and phasic EUS activity was observed during bladder contraction of 80% of VD+SH-TES animals, the EMG amplitude was decreased by ~ 90% compared to SH-VD. TES significantly increased the amplitude of EMG response during bladder contraction, however basal activity during intercontraction interval was not improved ($p<0.05$). At day 9 post-MBPdx, 20% of the MBPdx+SH-TES animals presented EUS EMG activity and 100% of the TES animals showed it. The amplitude of tonic and phasic discharge were greater in the stimulated group ($p <0.01$). The animals that were not stimulated showed low amplitude tonic activity.

Conclusions: Transinaptic activation of onuf´s neurons, via TES, facilitates recovery of EUS function in both, VD animals and direct nerve injury. Tonic and phasic activity recovered better in MBPdx than in VD group, probably due in the latter group the EUS fibers have not yet recovered. In rodents, tonic and bursting EUS motor units have been described. Our results indicate that both kinds of efferent axons are reconnected but tonic motor unit activity appears first, which suggest that there is an order in the axonal regeneration. This order could be related to different axonal diameter or to length distance from the axonal site of injury to neuron soma (where neurotrophic factors are produced). EUS bursting motor units are further away (lumbar levels) that tonic motor units (sacral segments), whereby could take more time for axonal transport of neurotrophic factors to the site of axonal reconstruction.

Funding Source(s): CONACYT Grant 183446 (YCG) and scholarship from CONACYT (RJM, 62305).

Title: The Relationship of Anterior Vesical Branches and Pelvic Ganglia to the Ureter in Human Cadavers

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Introduction/Objectives: We have demonstrated in human cadavers and dogs that nerve transfer to pelvic nerve vesical branches innervating the urinary bladder is technically feasible using a variety of donor nerves. Further clarification of the number and location of these nerves and the pelvic ganglia from which they arise is warranted. Our primary objective was to clarify in human cadavers, the relationship of pelvic nerve vesical branches and pelvic ganglia to the ureter at the site of its entrance into the bladder (i.e., ureteral orifice) to assist with identification during future surgeries. Our secondary objective was to examine sacral origins of pelvic nerve branches to the bladder.

Methods: Thirty-six pelvic regions were dissected in 24 human cadavers (17 females and 7 males, ages 56-97); 12 were studied bilaterally. Sixteen cadavers had been embalmed with formalin-phenol and 3 with Thiel fixative; 5 were unfixed. Formalin-phenol fixed cadavers were studied as an extension of routine anatomical dissection courses at Temple University. Thiel embalmed cadavers were part of a continuing medical education course at Saint Louis University. Unfixed cadavers were provided for the purpose of this study. In all cases, cadavers were inspected to ensure that the anatomy in the pelvic floor had not been damaged prior to our use. Gross dissection or laparoscopic methods were used. An anterior pelvic approach was used to identify the number of vesical nerve branches to the bladder, and anatomical location (and size) of pelvic ganglia in relation to the ureteral orifice in the bladder. Measurements were made using a caliper, flexible ruler, or a microscope after sectioning and staining of nerves and ganglia.

Results: The number of vesical branches from the pelvic ganglia to the bladder ranged from 1-4 in the 31 cadaveric sides examined (with a mean $\pm$ SD of 2.26 ± 0.44). The majority of pelvic ganglia were posterolateral to the ureteral orifice into the bladder (70%, 12 of 33 cadaver sides), although a few were located overlying the ureter at the ureteral orifice, or lateral, posterior, or anterolateral to the ureteral orifice. The average distance between the pelvic ganglia and ureteral orifice was 6.91 $\pm$ 6.30 mm, the diameter of the pelvic ganglia at its widest point was 3.90 $\pm$ 2.20 mm, and diameters of vesical branches were 2.37 $\pm$ 0.89 mm. The primary sacral origins of pelvic nerve branches to the bladder were from S2-4 (S2: 66.35% of cadavers, S3: 80.5%, S4: 47.75%). The most common combination was S2 and S3 (32%, 6/19 sides), and then S3 and S4 (26%, 5/19 sides). Two of 19 cadavers (10%) showed input from L5 and all sacral segments (L5, S1-S4).

Conclusions: The ureteral orifice is a clear landmark for locating the vesical branches of the pelvic nerve, as well as their associated pelvic ganglia. These nerve branches are sufficient in size for transfer of donor nerves for bladder reinnervation after sacral spinal cord injuries, e.g. sacral chordomas.

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Title: Wireless Monitoring of Colonic Activity in Ambulatory Untethered Animals

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Introduction/Objectives: Current devices for measuring or monitoring bowel function are only suitable for brief periods because they either pass through the viscus or they occlude the bowel and tether the patient. The goal of this work was to demonstrate a wireless sensor to measure colonic activity using the Colon Monitor to Capture Activity (ColoMOCA), which we developed.

Methods: The flexible ColoMOCA is sutured to the mucosal lining of the colon where it resides for several days before being passed out with feces. It wirelessly transmits data from 2 pressure sensors (63 mm apart, proximal and distal) and 3 impedance sensors. It is powered by a 3-mAh battery which supports wireless recharge and device activation after implant. During use, ColoMOCA data are wirelessly received by a wearable radio placed in the vest. The radio records data to an onboard SD card, and also transmits data over Bluetooth to a nearby computer. A ColoMOCA was implanted in each of 3 adult Yucatan minipigs. Daily data recording sessions of up to 2 hours of colonic activity began the day after implantation with animals fully conscious and untethered. During data recording the animal was given food and was able to walk, eat, urinate, and defecate freely. Data are shown as mean [interquartile range]. $P < 0.05$ indicates a significant difference between simultaneous proximal and distal pressures using a paired t-test.

Results: No animals showed signs of distress in response to the presence of the implanted ColoMOCA. Implanted ColoMOCAs remained patent and did not obstruct stool for 6-7 days of implantation. Devices were passed spontaneously through the gastrointestinal tract 6-7 days after implantation. Histology of explanted colon tissue did not show inflammatory response. Approximately 435 minutes of ambulatory, catheter-free colon pressure data from both ColoMOCA sensors were analyzed, from which 1,192 colonic contractions were identifiable. Implanted sensors showed a significant difference in contraction amplitudes between the proximal (5.1 [9.7] mm Hg) and distal (3.8 [6.7] mm Hg) pressure sensors ($p < 0.001$). Impedance measurements in conscious animals were qualitatively analyzed due to the novelty of this sensor modality. All recordings demonstrated periods of rhythmic impedance changes, followed by irregular, larger amplitude changes in impedance. Rhythmic slow waves had a period of approximately 1-3 minutes, consistent with previous reports on colonic activity.

Conclusions: ColoMOCA pressure waveforms were similar to those reported previously with colon pressure catheters. These initial data suggest that the ColoMOCA impedance sensing electrodes can detect stool movement, however, further validation studies are needed. The ColoMOCA could be useful to provide continuous data on colon activity in conscious ambulatory animals without tethers or catheters in preclinical trials of novel therapeutics for colonic dysfunction.

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Title: Catheter-free Bladder Volume Monitoring Using Intravesical Sensor

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Introduction/Objectives: Two key biomarkers of lower urinary tract function are bladder pressure and volume. Current measurement methods rely on catheters and do not reproduce natural bladder filling and emptying cycles. A wireless catheter-free sensor that continuously monitors bladder status would be an improvement over catheter-based devices. We have developed the Urological Monitor of Conscious Activity (UroMOCA), a surgically inserted wireless catheter-free pressure & volume bladder monitoring device. This work describes the *in vivo* performance of the UroMOCA) for continuous volume sensing.

Methods: The conductance of a fluid, such as urine, is proportional to the volume of its container. The UroMOCA has two active conductance-measuring electrodes with one common cathode. One closely spaced electrode pair measures conductivity, a property of the urine. Another electrode pair measures conductance, which corresponds to urine volume. Chronic survival UroMOCA implant studies were performed in 9 felines on an approved IACUC protocol. Animals were implanted with the UroMOCA under anesthesia via laparotomy and cystotomy. Following 1 week of post-op recovery, animals wore a jacket with a small radio that received bladder pressure and urine conductivity and conductance data transmitted from the UroMOCA. Bladder data, including spontaneous voiding events, were recorded while animals were awake and moving freely and untethered. Two and 4 weeks after implantation, anesthetized data recording sessions took place to evaluate device function with simultaneous cystometry. At the end of the 4 week procedure, the UroMOCA was surgically removed.

Results: Ambulatory data recording captured over 50 hours of catheter-free bladder recordings across all animals. Presence of the UroMOCA was not associated with adverse bladder function or animal disposition. Two UroMOCAs stopped transmitting 8 and 12 days after implantation, respectively; One UroMOCA transmitted intermittently for 2 weeks. The others transmitted bladder data through the entire experiment. Preliminary qualitative analysis showed that conductance measurements seemed to decrease during voiding and increase during bladder filling. In one randomly selected animal, the UroMOCA demonstrated noticeable volume decreases associated with 3 voiding events. This finding was supported by the simultaneous increase in UroMOCA pressure readings during those events.

Conclusions: The UroMOCA is a wireless, catheter-free platform technology that can be used for continuous monitoring of bladder pressure and volume in chronic experiments of awake, behaving animals. The ability to distinguish between voiding and non-voiding urinary events will aid neurophysiology studies of the lower urinary tract. Further analysis is required to quantify the UroMOCA's volume sensitivity.

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Title: Optimizing Human Activity Classification to Improve Monitoring and Treatment of Lower Urinary Tract Dysfunction

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Introduction/Objective: Implanted/inserted pressure sensors can enable long-term monitoring and detection of bladder events, enabling closed-loop stimulation for the treatment of lower urinary tract dysfunction (LUTD). Inertial measurement units (IMUs) can be leveraged to recognize human activity and postural transitions using machine learning (ML). Such motion tracking and subsequent activity classification can be integrated into a bladder event detection algorithm [1] to improve the classification of different bladder events and motion artifacts. This work investigates optimizing data from IMUs using different ML and signal processing techniques to reduce the power consumption and processing overhead required for activity classification.

Method: We utilized a human activity recognition dataset consisting of triaxial acceleration and angular velocity signals sampled at 50Hz. The dataset labels included six basic activities, three dynamic activities, and postural transitions [2]. Signals were processed to reduce noise and extract useful features for classifying activities. Initially, 561 features were extracted from the signals. To identify the features which had the greatest impact on classification accuracy, we explored three techniques: 1) Principal Component Analysis, 2) Random Forest (RF) Feature Importance, and 3) Random Projections. Once the top features were identified, we applied Gaussian Naive Bayes, Decision Tree, and Logistic Regression (LR) for activity classification. Training and testing were performed in a random 70-30 split.

Results: Using LR, the original classification accuracy using all 561 features was 96.1%. After the application of feature extraction and classification techniques, we found that the top 50 features identified by RF used in conjunction with an LR classifier achieved a detection accuracy of 93.9%, a 91% decrease in the feature space costing only a 2.2% decrease in accuracy. The top 10 features achieved an accuracy of 87.6% which consisted only of accelerometry-based features, without the need for a gyroscope. Hence, it is possible to significantly reduce the processing overhead for activity classification while minimally impacting the classification accuracy.

Conclusion: In conjunction with automated bladder event detection, human activity classification using IMU data can potentially improve catheter-free, long-term monitoring and treatment of LUTD. In this work, we demonstrate how feature reduction techniques and ML classifiers can be used to optimize the classification of human activities with minimal impact on accuracy.

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Title: Sensory Neuropeptides Modulate the ATP Hydrolysis by Soluble Nucleotidases in the Urinary Bladder Lamina Propria

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Introduction/Objectives: Adenosine 5'-triphosphate (ATP) is an important signaling molecule in the suburothelium (lamina propria, LP) that activates P2X2/X3 receptors on sensory neurons in LP and initiates voiding. Pituitary adenylate cyclase-activating polypeptide 1-38 (PACAP38), substance P (Sub P), and calcitonin gene-related peptide (CGRP) are sensory mediators that increase bladder afferent nerve activity and release ATP from urothelial cells. Soluble nucleotidases (sNTDs) are released in the LP in a mechanosensitive manner and contribute significantly to the extracellular ATP hydrolysis to ADP, AMP, and adenosine (ADO). It is presently unknown whether sensory neurotransmitters modulate the release of sNTDs, and hence the degradation of ATP in the LP.

Methods: Using ultrasensitive HPLC methodologies, we measured the degradation of 1N^6-etheno-ATP substrate (eATP, 2 μM), to eADP, eAMP, and eADO in solutions that were previously in contact with the LP of nondistended (empty) or distended (full) murine detrusor-free bladder preparations. The degradation of eATP by sNTDs was evaluated in concentrated extraluminal solutions (cELS) without tissue present, in the absence or presence of agonists (e.g., PACAP38, Sub P, and CGRP) and antagonists (e.g., PACAP6-38, CP12272 and BIBN 4096) of PAC1, NK1, and CGRP receptors, respectively.

Results: eATP was hydrolyzed to eADP, eAMP and eADO by sNTDs. One hour after adding eATP to cELS from nondistended preparations, eATP was diminished from 94.11±1.00% to 58.09±5.2 % of total purines in controls (n=9), and to 26.34±2.57% (n=3, $P<0.05$ vs. controls), 26.59±4.24% (n=3, $P<0.0001$ vs. controls), and 18.03±4.19% (n=3, $P<0.001$ vs. controls) in the presence of PACAP38 (100 nM), Sub P (1 μM) and CGRP (100 nM), respectively. In the cELS from distended preparations, eATP was decreased from 93.98±0.64% to 32.73±5.68% in controls and to 7.51±2.75% in the presence of CGRP ($P<0.05$ vs. controls). PACAP38 and Sub P, however, did not alter the distention-induced release of sNTDs. The receptor antagonists PACAP6-38 (300 nM), CP12272 (1 μM), and BIBN 4096 (1 μM) had lesser effects than the exogenous receptor agonists on the degradation of eATP by sNTDs in cELS from undistended and distended preparations.

Conclusions: The observed discrepancy between the effects of endogenous and exogenous neuropeptides suggests that neither the constitutive nor the mechanosensitive release of sNTDs is modulated by physiological levels of sensory neuropeptides in the LP. However, elevated concentrations of PACAP38, Sub P or CGRP, as anticipated to occur in bladder inflammation, bladder pain syndrome, and overactive bladder, would be expected to increase the constitutive release of sNTDs and to facilitate the degradation of extracellular ATP. The neuropeptide-enhanced ATP hydrolysis would counteract the hyperexcitability of the bladder caused by increased levels of extracellular ATP in such conditions. Neuropeptide modulation of nucleotidase release is a novel mechanism of regulation of bladder excitability during filling.

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Title: TRPM8 channels activation induces relaxation in pudendal arteries and corpus cavernosum from diabetic mice

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Introduction/Objectives: Diabetes mellitus is a chronic metabolic disease with epidemiological relevance worldwide. One of the most common and most underestimated complications of diabetes is erectile dysfunction (ED). Previous studies have shown that TRPM8 activation triggers relaxation of internal pudendal arteries (IPA) with increased sensitivity in the hypertensive rats. However, despite the correlation between ED and diabetes, research on the role of TRPM8 channels in the vasculature responsible for sexual function in diabetic condition still needs to be elucidated. In this way, the aim of this study was to investigate TRPM8 channels in IPA and corpus cavernosum (CC). We hypothesized that TRPM8 activation would induce relaxation in IPA and CC from diabetic mice, which may help to improve erectile function.

Methods: Male lean and db/db mice were euthanized, and the IPA were mounted on DMT wire myographs and CC were mounted on AVS organ bath for the measurement of isometric force. Concentration-response curves for relaxation to icilin (10^{-8} to $3x10^{-4}$M) and menthol (10^{-6} to 10^{-3}M) were obtained. Expression of TRPM8 channels was evaluated by western blot using CC. Data are expressed as mean ± S.E.M of 4-5 mice.

Results: TRPM8 channels are expressed in the CC of lean and db/db mice and revealed equivalent expression between these mice. Menthol, a TRPM8 agonist, promoted relaxation in pre-contracted CC strips with phenylephrine (E_{max}: 139.44 ± 15.91%) in lean mice. Similarly, the relaxation induced by menthol was not changed in the CC from db/db mice (E_{max}: 105.00 ± 5.00%*).* Cumulative administration of icilin, a TRPM8 agonist, induced endothelium-dependent and endothelium-independent relaxation in pre-contracted rings IPA with phenylephrine. Relaxant effect induced by icilin was similar between IPA from db/db (E_{max}: 95.74 ± 0.96%) and lean (E_{max}: 97.05 ± 0.87%) mice. Diabetes increased the contractility of the IPA in response to phenylephrine in concentration of $3x10^{-6}$M (99.40 ± 4.65%) compared to lean (85.44 ± 2.36%). Interestingly, pre-incubation with icilin (10^{-4}M) decreased the potency of the contraction induced by phenylephrine (10^{-9} to $3x10^{-5}$M) compared to untreated rings IPA in both db/db (pD_2: 6.39±0.07 vs 6.67±0.05) and lean (pD_2: 6.43±0.09 vs 6.82±0.05) mice. In the IPA, diabetes decreased the sensitivity to acetylcholine compared to lean (pD_2: 7.17±0.06 vs 7.42±0.03). However, the relaxation induced by acetylcholine was not changed by pre-incubation with icilin.

Conclusions: TRPM8 agonists are able to promote relaxation with the same magnitude in IPA and CC from diabetic and lean mice. Increased responses to phenylephrine in IPA may contribute to the development of ED. In addition, pre-incubation with icilin reduced sensitivity to phenylephrine, suggesting that the icilin could be a helpful treatment for ED.

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Urothelial Response to Short-term and Long-term Hypoxic Conditions

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Introduction/Objectives: Bladder outlet obstruction (BOO) affects around 18.5% of the population and is caused by a narrowing of urethra.[1] The obstruction results in mechanical changes in the bladder such as elevated pressure and blood flow disruptions that causes tissue ischemia.[2,3] These changes can lead to chronic inflammation in the urothelium. Our previous study demonstrated that exposure to elevated hydrostatic pressure (40 cmH2O for 1 min) led to ATP-mediated NLRP3 inflammasome activation in rat urothelial cells *in vitro.*[4] Additionally, studies have shown that 48 hours of hypoxic exposure can cause increased inflammatory cytokine release in bladder smooth muscle cells.[5] However, the role of for hypoxia in BOO-induced inflammation in the urothelium is unknown. Thus, we examined the short-term and long-term effects of hypoxia on NLRP3 activation in rat urothelial cells (MYP3) cells using a hypoxic culture media containing enzymes glucose oxidase (GOX) and catalase (CAT).

Methods: MYP3 cells were exposed to *in vitro* hypoxic conditions via a two-enzyme system for 2 or 6 hours. To validate the results obtained using the enzyme-based hypoxia model, a modular hypoxia chamber (flushed with a gas composed of 5% O_2, 5% CO_2 and balanced with nitrogen) was also used. The hypoxic media was prepared with 0.5 µg/mL GOX in phenol-red free F-12K media (VWR) with catalase (CAT, 120 U/mL) and HEPES (25 mM) to remove hydrogen peroxide, and to buffer pH, respectively. MYP3 hypoxic response was examined through HIF-1α stabilization via immunostaining of the cells and nitric oxide (NO) release in the supernatant measured using Griess assay (Fisher). Inflammasome activation was quantified through intracellular capsase-1 via an established protocol.[4] Numerical data were statistically analyzed with GraphPad Prism 5 using either one-way or two-way analysis of variance (ANOVA). P-values less than 0.05 were considered statistically significant.

Results: In the short-term (up to 2 hours), both enzyme and gas-induced hypoxia caused a significant increase in NO levels by MYP3 cells after 1 hour when compared to normoxic conditions. However, exposing MYP3 cells to hypoxia through neither method led to NLRP3 activation as no increase in intracellular caspase-1 levels were observed. In contrast, exposure to long-term hypoxia (up to 6 hours) resulted in about a 1.6-fold increase in intracellular caspase-1 activity in MYP3 cells compared to control. These results indicate that longer exposure is needed to elicit an inflammatory response in urothelial cells even though with shorter time periods, a hypoxic response was observed.

Conclusion: Although elevated pressure rapidly stimulated ATP-mediated NLRP3 inflammasome in urothelial cells after 1 min[4], the results of the present study indicate hypoxia-induced inflammasome activation requires longer time periods and may be driven by another pathway such as ROS-TXNIP axis. Further work is needed to elucidate the mechanisms behind hypoxia induced inflammation in urothelial cells as it relates to BOO.

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Title: Characterization of cardiovascular function, metabolic status and erectile function in rat model of cardiometabolic syndrome

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Introduction/Objectives: Cardiometabolic syndrome is a widespread health issue and common cause of erectile dysfunction (ED), yet the molecular basis of ED in cardiometabolic syndrome needs to be elucidated. Our aim is to characterize the cardiovascular and erectile function, and metabolic status in the obese ZSF-1 rat. This hybrid rat is a cross between a Zucker diabetic fatty female and a spontaneously hypertensive heart failure male rat.

Methods: We used male lean and obese ZSF-1 rats (n=8/group). We measured body weight weekly, and performed MRI and glucose tolerance tests at 10, 20, 30 weeks. At 30 weeks, we assessed erectile function using ICP/MAP and vascular stiffness was measured via laser doppler pulse wave velocity (PWV). Additionally, tissue bath experiments measured acetylcholine (Ach)-mediated vasodilation, vasodilation to sodium nitroprusside (SNP), and contraction to phenylephrine (PE) in thoracic and abdominal aortas. Aorta and penile segments were collected for histological analyses.

Results: On average, obese rats were 60% larger (230g) than controls and had significantly impaired glucose tolerance at all time points ($p<0.05$). At 30 weeks, obese ZSF-1 rats had significantly reduced ICP/MAP compared to controls indicating the development of erectile dysfunction ($p<0.05$). Obese rats were significantly more hypertensive compared to lean rats (MAP: 140.2 mmHg vs 99.6mmHg, $p<0.05$). Lean and obese rats had no difference in vascular stiffness indicated by PWV. In our tissue bath experiments, ACh-mediated vasodilation was significantly decreased in the thoracic aorta of obese rats ($p<0.05$) while the abdominal aorta was unchanged. SNP-mediated vasodilation was similar between groups in both thoracic and abdominal aortas. At high concentrations, PE-mediated vasoconstriction was significantly increased in the thoracic and abdominal aortas of obese rats ($p<0.05$).

Conclusions: To our knowledge, this is the first report on the erectile function in the ZSF-1 rat model of cardiometabolic syndrome. We believe that the ZSF-1 rat is useful for studying erectile dysfunction as they gradually develop metabolic disease and erectile dysfunction over time similar to the human disease. Further characterization of the penile smooth muscle physiology, and penile and aortic histology will follow.

Funding Source(s): DiaComp Pilot and Feasibility Grant Charles River Animal Model Evaluation Program

Title: Specialized pro-resolving mediator Resolvin E1 improves diabetic bladder dysfunction in male Akita mice

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Introduction/Objectives: >50% of patients with diabetes suffer from diabetic bladder dysfunction (DBD), which produces a wide variety of urinary symptoms including underactive bladder. Current treatments (glycemic control, pharmacotherapy and surgery) are ineffective creating an unmet need for novel targeted therapies. Previous studies using female Akita Type 1 diabetic mice implicate the NLRP3 inflammasome in the development of an overactive phase of DBD. Inflammation is a balance between initiation (through NLRP3) and resolution (through specialized pro-resolution mediators such as Resolvin E1 (RvE1)) and since most patients present with DBD already established, we sought to explore the therapeutic potential of stimulating the resolution phase of inflammation. We hypothesize that RvE1 treatment in male Akita mice at 15 weeks of age, which develop underactive DBD, will improve bladder function assessed by urodynamics.

Methods: Suprapubic tubes were placed in control and male Akita at 15 weeks of age. Mice then received RvE1 (25 μg/kg daily, i.p.) for 7 days and cystometry was performed. Various parameters (void volume, frequency, etc.) were then analyzed. Data was analyzed using ANOVA followed by Student-Newman-Keul's post hoc test.

Results: Void volume and bladder capacity was increased in untreated diabetic mice while voiding frequencies were decreased, consistent with an underactive bladder. Excitingly these parameters were significantly, albeit not completely, reversed by RvE1. This suggests that, after only 1 week, RvE1 can have therapeutic effects on DBD. Future studies will increase the length of treatment to determine if more complete restoration of function is possible. Intuitively, one would propose the beneficial effect of Resolvin E1 is due to a reduction in inflammation. However, results presented in a separate abstract demonstrate that the genetic deletion of NLRP3 in these mice prevents inflammation, as measured by Evans blue extravasation, yet underactivity still develops. Consequently, Resolvin E1 must be acting on non-inflammatory pathways, or inflammatory pathways not reflected by the Evans blue dye extravasation assay, to trigger this remarkable recovery of bladder function. Future studies will explore such pathways.

Conclusions: Resolvin E1 administration for one week improved urinary function in 15 week old male Akita diabetic male mice with bladder underactivity.

Funding Source(s): RO1 DK117890.

Title: Expression of an engineered chloride channel reduces bladder overactivity and inflammation in rats with cyclophosphamide-induced cystitis.

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Introduction/Objectives: Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a disease of the urinary bladder that causes increased urgency to void and pelvic pain. It is thought that increased bladder sensations in IC/BPS are due to sensitization of bladder afferent nerves by inflammatory mediators. Current therapies aimed at known targets in the inflammatory or nociceptive pathways have, to date, been ineffective at reducing the symptoms in IC/BPS patients. To find potential new treatments for IC/BPS, we have attempted to express a non-native chloride channel (named EG3RF) in bladder afferent neurons. This channel can be activated by either inflammatory conditions or by an exogenous pharmaceutical compound (cysteamine) but has no activity under normal conditions. It is our hope that this channel, when expressed in bladder afferent nerves, will be activated by inflammation in an animal model of IC/BPS (intraperitoneal cyclophosphamide), preventing their sensitization and thus eliminating the bladder overactivity normally exhibited in the model.

Methods: A plasmid containing the sequence for the EG3RF chloride channel was encapsulated in liposomes and instilled into the urinary bladder of Sprague Dawley rats using a transurethral catheter. One to two weeks later, bladder inflammation was induced using an intraperitoneal injection of cyclophosphamide (CYP, 150mg/kg) 24 hours before metabolic cage recordings or urethane anesthetized bladder cystometry was performed. Bladder edema was measured using plasma extravasation of Evans Blue dye. Successful transfection of tissue was confirmed by collection of the bladder smooth muscle, urothelium and L6-S1 dorsal root ganglia and the extraction of mRNA for PCR.

Results: PCR amplification demonstrated that intravesical transfection of the EG3RF plasmid resulted in receptor mRNA expression in the urothelium, detrusor and L6-S1 DRGs up to 2 weeks after instillation. Metabolic cage or cystometry experiments indicated no differences in voiding function between EG3RF transfected and control animals. However, EG3RF-expressing rats were resistant to the excitatory effects of cyclophosphamide treatment. Cysteamine treatment decreased bladder activity in both metabolic cage and cystometry experiments (31mg/kg ip or iv, respectively) in CYP treated rats but was ineffective in normal rats. EG3RF transfected rats also exhibited much less bladder edema in response to CYP than non-transfected rats.

Conclusions: Intravesical instillation of liposome encased EG3RF plasmids was successful in transfecting multiple bladder tissues. EG3RF expression alone prevented the excitatory effects of CYP on reflex bladder activity, and treatment with EG3RF's agonist cysteamine further decreased voiding frequency. EG3RF also reduced bladder inflammation, suggesting that expression of our engineered channel could treat IC/BPS by multiple mechanisms.

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Title: Differential expression of myosin 5a isoforms in visceral organ innervation.

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Introduction/Objectives: The unconventional motor protein, Myosin 5a (Myo5a) is enriched in neural tissues, where it is involved with transport of synaptic vesicles across the cortical actin network for neurotransmitter secretion. A segment of the Myo5a tail domain (exons A, B, C, D, E and F) can undergo alternative splicing to produce variants that may contain one or more optional exons (B, D, F) along with exons which are always present (A, C, E). The distribution and function of individual Myo5a variants are, for the most part, unknown. We hypothesized that diverse Myo5a variants would be found in peripheral nerves, where they would serve specific functional roles depending on the tissue innervated and their subcellular localization. In this work, we have identified the Myo5a variants expressed in several visceral tissues (pylorus, detrusor, bladder mucosa, urethra, corpus cavernosum, and major pelvic ganglion).

Methods: Total RNA from each tissue was extracted, converted to cDNA, and used for PCR with primers spanning the alternative exon region. Initial products were used as templates for nested PCR with internal primers. Size and restriction enzyme digestion patterns were determined by agarose gel electrophoresis versus molecular size markers. Quantitative PCR (RT-PCR) was performed with primer/probe sets recognizing exon B, exon F or all variants.

Results: In the pylorus, a tissue comprising neuronal cell bodies as well as axons, all three optional exons (B, D and F) were detected. When analysis was extended to the major pelvic ganglion (MPG) and to urogenital tissues without intramural ganglia (bladder, mucosa, urethra corpus cavernosum), exon B was found only in the plexus and, to a minor extent, in the urethra. In contrast, exons D and F predominated in several configurations in all tissues except the MPG.

Conclusions: This study indicates that the ABCE splice variant of Myo5a is present primarily in the soma/dendrites of peripheral nerves, whereas other variants (ACDE, ACEF, ACDEF) predominate in axonal projections within visceral organs. This selective expression of splice variants may underly a differential functional role for myosin 5a in the soma in contrast with the axon, whereas the variable extent of expression of non-exon B-containing variants in visceral organs may allow interactions with particular synaptic cargos to meet the specific neurotransmitter trafficking requirements of each organ.

Funding Source(s): Department of Veterans Affairs, Biomedical Laboratory R&D, BX001790

Title: Targeted depletion of Fidgetin-like 2 (FL2) promotes cavernous nerve regeneration and improves erectile and bladder function outcomes in a rodent model of radical prostatectomy.

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Introduction/Objectives: Damage to the cavernous nerves (CN) during radical prostatectomy (RP) often causes urogenital dysfunction, including bladder and erectile dysfunction (ED). We recently demonstrated that the microtubule regulatory protein, fidgetin-like 2 (FL2), can be targeted to promote wound healing and promote nerve regeneration in a rodent model of RP. We have expanded upon these recently studies to determine if FL2 depletion might also improve bladder function outcomes following CN injury.

Methods: As a model of severe CN injury, rats (3-4 months) underwent CN transection. At the time of injury, control- or FL2-siRNA was applied as a wafer-like formulation directly to the site of CN injury. Regeneration of the CN was assessed visually 2-weeks after injury and erectile function was assessed by measuring the intracorporal pressure/blood pressure (ICP/BP) ratio following electrostimulation of the CN. Voiding function was evaluated one-week post-injury, using the voided stain on paper (VSOP) method. To study the effects of FL2 depletion on axon growth and on the microtubule (MT) cytoskeleton within the axon shaft *in vitro*, dissociated adult rodent dorsal root ganglion (DRG) neurons were transduced with AAV5 containing a plasmid encoding FL2 or scrambled small hairpin RNA (shRNA) and a GFP reporter. Five-days after treatment, neurons were replated for live monitoring of neurite growth, fixed and immunostained for MT.

Results: Animals treated through application of FL2-siRNA-wafer at the site and time of bilateral transection displayed significantly lower voiding frequency and higher voided volume per micturition when compared to naïve (unoperated) age-matched controls. There was visible regeneration of the CN in 7 out of 8 animals treated with FL2-siRNA (with no observed regeneration in animal treated with control-siRNA). In animals in which there was sufficient CN regeneration to allow electrostimulation, FL2-siRNA treated animals had significantly higher ICP/BP ratios compared to controls. *In vitro,* knockdown of FL2 in neurons enhanced average neurite length and growth rate. Analysis of MT subpopulations by quantitative immunofluorescence indicated an increase in the ratio of dynamic to stable MTs in the axon shaft with FL2 depletion.

Conclusions: FL2-depletion after CN injury results in visible regeneration of the CN, with improved erectile and bladder function outcomes. Our work suggests that depleting FL2 promotes nerve regeneration through directly enhancing the growth rate of injured axons, possibly by increasing the amount of dynamic MTs in the distal axon. Overall, we have identified FL2 as a promising therapeutic target for promoting CN regeneration after injury, such as occurs during the surgical procedures of RP.

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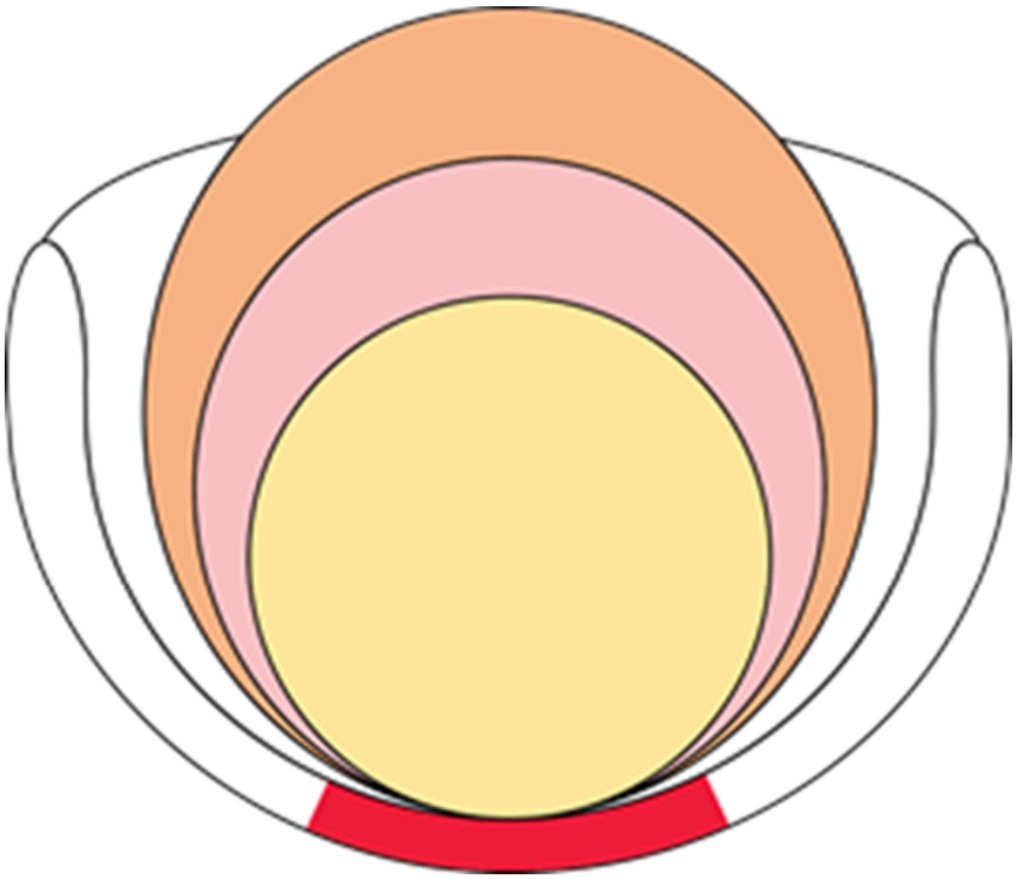

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