THE SOCIETY FOR PELVIC RESEARCH

THIRD ANNUAL MEETING

MEETING PROGRAM

November 30 - December 2, 2018
New Orleans, LA

#PelvRes18
Sponsored by:

Generous donations by the Glickman Urological & Kidney Institute at Cleveland Clinic, Duke University Division of Urology and Medtronic

In Partnership with:

The International Continence Society

Special Thanks to:

Ms. Elizabeth Foss CTC, MCC, MBA, President of My Travel Elf, Inc. / MTE Vacations, Naples, FL. As was the case with SPR 2016 and 2017, Beth played a pivotal role as an advisor and in organizing the venue, food and beverages, audiovisual, poster boards, etc., and the contracts involved with this event. She has once again generously donated her time and expertise.

The 2018 SPR Abstract Review Committee
  Brian M. Balog, PhD Candidate
  Kelvin P. Davies, PhD
  Michael E. DiSanto, PhD
  Carol A. Podlasek, PhD
  Maryrose P. Sullivan, PhD
  Matthew O. Fraser, PhD

The 2018 SPR Trainee Award Judges
  Kelvin P. Davies, PhD
  Robert A Gaunt, PhD
  Johanna L. Hannan, PhD
  Anna S. Nagle, PhD
  Carol A. Podlasek, PhD
  Michael R. Ruggieri, Sr, PhD
  Maryrose P. Sullivan, PhD
  Sean M. Ward, PhD
  Matthew O. Fraser, PhD

The 2018 SPR Local Support Volunteers
  Maureen E. Basha, PhD
  Joanna M. Togami, MD
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 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.

The Society for Pelvic Research was born.

The Society For Pelvic Research is a North Carolina Non-Profit Corporation that filed on May 12, 2015. The company's filing status is listed as Current-Active and its File Number is 1444909.

Tax exempt status under Internal Revenue Code (IRC) Section 501(c)(3) was granted effective May 12, 2015. Donors can deduct contributions under IRC Section 170. The Society may accept tax deductible bequests, devises, transfers or gifts under Sections 2055, 2106 Or 2522.

The Society for Pelvic Research Public Charity Status is 509(a)(2).

This year’s meeting represents the cumulative efforts of our board, volunteer members, advisors and funders over the past year. We are already looking forward to next year’s meeting.
Our Board of Directors

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Associate Professor, Department of Surgery, Division of Urology, Duke University and Durham VA Medical Centers

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Lerner Research Institute at the Cleveland Clinic and The University of Akron

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Georgi V. Petlov, PhD
Professor and Chair, Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, College of Pharmacy

Michael R. Ruggieri, Sr, PhD
Associate Professor, Department of Anatomy and Cell Biology, Temple University School of Medicine
Program Summary

November 30, 2018
7:00 PM    Trainee Affairs Committee Workshop - Kimberly Putman, PCC, ICF
9:00 PM    Trainee Social Event

December 1, 2018
7:00 AM    Continental Breakfast / Trainee Breakfast with the Experts
    Experts: Dr. Dolores J. Lamb, PhD, Dr. Francesco Demayo, PhD, Dr. Patricia Greenwell, PhD, and Dr. Kenton M. Sanders, PhD
8:00 AM    Welcome, Opening Remarks, Mission of the SPR - Matthew O. Fraser, PhD
8:10 AM    Session 1: Neurotrauma and Pelvic Function
    Moderators: Michael R. Odom, PhD Candidate and Dennis J. Bourbeau, PhD
    Key Note Speaker - Dr. Kim D. Anderson-Erisman, PhD
    Q&A
    Oral Presentations - Abstracts S1A1-S1A5
    Q&A
10:10 AM    Break
10:25 AM    Session 2: Muscle and Organ Function
    Moderators: Aileen Ouyang, PhD Candidate and Michael R. Ruggieri, Sr, PhD
    State of the Art Speaker/International Continence Society Lecturer
        - Dr. Karen D. McCloskey, PhD
    Q&A
    Oral Presentations - Abstracts S2A6-S2A10
    Q&A
12:25 PM    Lunch
1:25 PM    Session 3: Models and Methods
    Moderators: Laith Alzweri, MD, Urology Fellow and Maryrose P. Sullivan, PhD
    State of the Art Speaker - Dr. Tim M. Bruns, PhD
    Q&A
    Oral Presentations - Abstracts S3A11-S3A15
    Q&A
3:25 PM    Break
3:40 PM    Session 4: Special Topics—NIDDK GI Program Director
    Moderators: Danielle M. Salvadeo, MD/PhD Student and Sean M. Ward, PhD
    Special Guest Speaker - Dr. Patricia Greenwel, PhD
    Q&A
4:20 PM    Break
5:00 PM    Poster Session / Reception
    Moderators: Carol A. Podlasek, PhD, Anna S. Nagle, PhD, and Robert A. Gaunt, PhD
    Poster Presentations - Abstracts PS31-PS48
7:00 PM    Adjourn for the Day

December 2, 2018
7:00 AM    Continental Breakfast
8:00 AM    Welcome to Day 2 - Sean M. Ward, PhD
8:10 AM    Session 5: Fertility / Sexual Function
    Moderators: Brian M. Balog, PhD Candidate and Dr Suresh C. Sikka, PhD, HCLD
    Key Note Speaker - Dr. Dolores J. Lamb, PhD, HCLD
    Q&A
# Program Summary

**December 2, 2018**

9:10 AM  **Session 5: Fertility / Sexual Function (Continued)**  
   **Oral Presentations** - Abstracts S5A16-S5A20  
   Q&A

10:10 AM  **Break**

10:25 AM  **Session 6: Physiology and Pharmacology**  
   **Moderators:** Ekta Tiwari, PhD Candidate and Johanna L. Hannan, PhD  
   **Special Guest Speaker** — **Dr. Francesco J. Demayo, PhD**  
   Q&A  
   **Oral Presentations** - Abstracts S6A21-S6A25  
   Q&A

12:25 PM  **Lunch**

1:25 PM  **Session 7: Novel Therapeutics / Diagnostics**  
   **Moderators:** Gabrielle L. Clark, PhD Student and Kelvin P. Davies, PhD  
   **Oral Presentations** - Abstracts S7A26-S7A30  
   Q&A

2:25 PM  **Break**

2:40 PM  **Awards and Closing Remarks**  
   **Awards:** Matthew O. Fraser, PhD and Sean M. Ward, PhD  
   **Closing Remarks:** Matthew O. Fraser, PhD

3:15 PM  **Meeting Adjourns**

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#PelvRes18
Program in Detail

November 30, 2018

Trainee Affairs Committee Workshop
7:00 PM 1:00 Workshop Leader: Kimberly H. Putman, PCC, ICF
“Mastering the Interview Process”
9:00 PM 2:00 Trainee Social Event

December 1, 2018
7:00 AM 1:00 Continental Breakfast/Trainee Breakfast with the Experts
Experts: Dolores J. Lamb, PhD, Francesco Demayo, PhD, Patricia Greenwell, PhD, and Kenton M. Sanders, PhD
8:00 AM 0:10 Welcome, Opening Remarks, Mission of the SPR
SPR President: Matthew O. Fraser, PhD

Session 1: Neurotrauma and Pelvic Function
Moderators: Michael R. Odom, PhD Candidate and Dennis J. Bourbeau, PhD
8:10 AM 0:50 Key Note Speaker: Kim D. Anderson-Erisman, PhD
"Pelvic Dysfunction after SCI: Data from the Lived Experience"
9:00 AM 0:10 Q&A
Oral Presentations - Abstracts S1A1-S1A5
9:10 AM 0:10 S1A1 Danielle M. Salvadeo, MD/PhD Student - Assessment of bladder function after year-long lower spinal root transection and surgical reinnervation using behavioral measures
9:20 AM 0:10 S1A2 Guillermo A. Villegas, PhD - Inflammation in Development of LUTS following Radical Prostatectomy
9:30 AM 0:10 S1A3 Brian M. Balog, PhD Candidate - BDNF is Essential for Pudendal Nerve Motor Branch Functional Recovery
9:40 AM 0:10 S1A4 Dale R. Sengelaub, PhD - Neuroprotection of sphincter motoneurons after spinal cord injury
9:50 AM 0:10 S1A5 Bradley A. Potts, MD - Striking Differences in the Effects of 83-Adrenoceptor Agonists and Antimuscarinics on Bladder Filling/Voiding Function in Chronic Spinal Cord Injured Rats
10:00 AM 0:10 Q&A
10:10 AM 0:15 Break

Session 2: Muscle and Organ Function
Moderators: Zhonghua (Aileen) Ouyang, PhD Candidate and Michael R. Ruggieri, Sr, PhD
10:25 AM 0:50 State of the Art Speaker/International Continence Society Lecturer:
Karen D. McCloskey, PhD
"Emerging Concepts in Bladder Physiology - New Targets for Translation?"
11:15 AM 0:10 Q&A
Program in Detail

December 1, 2018

Session 2: Muscle and Organ Function (Continued)

Moderators: Zhonghua (Aileen) Ouyang, PhD Candidate and Michael R. Ruggieri, Sr, PhD

Oral Presentations - Abstracts S2A6-S2A10

11:25 AM  0:10  S2A6  Sean M. Ward, PhD - Calcium waves and spatio-temporal mapping reveals myosalpinx excitability and propagation along the murine oviduct

11:35 AM  0:10  S2A7  Tong Zhou, PhD - Transcriptomic analysis of bladder PDGFRα+ cells in early diabetic bladder

11:45 AM  0:10  S2A8  Rosalyn M. Adam, PhD - Neuropilin 2: a novel regulator of gut motility

11:55 AM  0:10  S2A9  Violeta N. Mutafova-Yambolieva, MD PhD - Basal and filling-induced degradation of adenosine 5’- triphosphate (ATP) at suburothelium/lamina propria of the murine bladder

12:05 PM  0:10  S2A10  Caroline A. Cobine, PhD - A unique population of PDGFRα+ cells are expressed in the Cynomolgus monkey internal anal sphincter

12:15 PM  0:10  Q&A

12:25 PM  1:00  Lunch

Session 3: Models and Methods

Moderators: Laith Alzweri, MD, Urology Fellow and Maryrose P. Sullivan, PhD

1:25 PM  0:50  State of the Art Speaker: Tim M. Bruns, PhD

"Neuromodulation for Bladder and Female Sexual Dysfunction: Tapping into Sensory Pathways"

2:15 PM  0:10  Q&A

Oral Presentations: Abstracts S3A11-S3A15

2:25 PM  0:10  S3A11  Ektaa Tiwari, PhD Candidate - Improved electrophysiological techniques to monitor hypogastic nerve activity during bladder filling in canines

2:35 PM  0:10  S3A12  Aref Smiley, PhD - Wireless Real-Time Sensor Platform for Monitoring Bowel State and Function

2:45 PM  0:10  S3A13  Sung Jin Hwang, PhD - Progerin causes disruption in enteric neurons and Interstitial cells of Cajal in the colons of Imna^hg-c mice

2:55 PM  0:10  S3A14  Damiano Angoli, PhD - Weeks-Long Continuous Monitoring of Rodent Urodynamic Parameters Including Post Void Residual Volumes using a Novel Hybrid Physical-Computational Metabolic Cage System

3:05 PM  0:10  S3A15  Zhonghua (Aileen) Ouyang, PhD Candidate - Behavioral monitoring and neuromodulation of feline bladder function

3:15 PM  0:10  Q&A

3:25 PM  0:15  Break
Program in Detail

December 1, 2018

Session 4: Special Topic – NIDDK GI Program Director

Moderators: Danielle M. Salvadeo, MD/PhD Student and Sean M. Ward, PhD

3:40 PM 0:30 Special Guest Speaker: Patricia Greenwel, PhD
“Basic and Translational Research Funding Opportunities from NIDDK/DDN”

4:10 PM 0:10 Q&A

4:20 PM 0:40 Break

Poster Session / Wine and Cheese Reception

Moderators: Carol A. Podlasek, PhD, Anna S. Nagle, PhD and Robert A. Gaunt, PhD

5:00 PM 2:00 Poster Presentations: Abstracts PS31-PS48

PS31 Michael R. Odom, PhD Candidate - Comparison of systemic and penile vascular function in C57Bl/6N and C57Bl/6J mice following 12 weeks of high fat diet

PS32 Shataakshi Dahal, PhD - Quantitative morphometry assessment of elastic fibers in pelvic organ prolapse

PS33 Akinjide R. Akintunde, PhD Candidate - Effects of elastase digestion on the murine vaginal wall biaxial mechanical response

PS34 Bernard T. Drumm, PhD - Tonic inhibition of murine proximal colon contractions is due to nitrenerg suppression of Ca2+ release events coupled to activation of Ano1 in interstitial cells of Cajal

PS35 Sarah Maxwell, PhD Student - The b-adrenoceptor agonist isoproterenol attenuates detrusor smooth muscle cell excitability and spontaneous contractility by activating Kv7 channels in guinea pig urinary bladder

PS36 Moses T. Tar, MD - Impact of nanotechnology in the post-surgical treatment of infection and inflammation in penile implants

PS37 Gabrielle Clark, PhD Candidate - Biaxial Contractile Response of Murine Vaginal Tissue

PS38 Alan S. Braverman, PhD - Nicotinic receptors on nerve terminals induce acetylcholine but not ATP release in canine bladder

PS39 Laith Alzweri, MD, Urology Fellow - Eugonadal Testosterone Levels Positively Regulates Erectile Function in Isolated Human Corpus Cavernosum

PS40 Laith Alzweri, MD, Urology Fellow - Collagenase clostridium histolyticum (CCH) attenuates human corpus cavernosum contraction in vitro

PS41 Lisa M. Jungbauer Nikolas, PhD - Ovine Model for Preclinical Urological Research: Extending the Large Animal Model to Bladder Hyperactivity?

PS42 Tong Zhou, PhD - Expression profile of platelet-derived growth factor receptor α (PDGFRα)-mediated genesets differentiates interstitial cystitis

PS43 Steve J. A. Majerus, PhD - Automated closed-loop stimulation to inhibit neurogenic bladder overactivity

PS44 Lisa A. Baker, PhD Candidate - Targeting Fidgetin-like 2 to rescue locomotor and lower urogenital function after spinal cord injury
Program in Detail

December 1, 2018

Poster Session / Wine and Cheese Reception (Continued)

Moderators: Carol A. Podlasek, PhD, Anna S. Nagle, PhD, and Robert A. Gaunt, PhD
5:00 PM 2:00 Poster Presentations: Abstracts PS31-PS48

PS45 Nagat Frara, PhD - Extensive sensory decentralization attenuates adenosine triphosphate (ATP) release from bladder intramural nerve endings in canines

PS46 Danielle M. Salvadeo, MD/PhD Student - Bladder smooth muscle strip contractility studies are reliable up to 48 hours after harvest

PS47 Wenbin Yang, PhD - Lipid Modulation of Pelvic Pain

PS48 Gabrielle Clark, PhD Candidate - Effect of Parity on Murine Vaginal Wall Elastic Fiber Structure and Mechanical Function

7:00 PM 0:00 Adjourn for the Day

December 2, 2018

7:00 AM 1:00 Continental Breakfast

8:00 AM 0:10 Welcome to Day 2

Vice President: Sean M. Ward, PhD

Session 5: Fertility / Sexual Function

Moderators: Brian M. Balog, PhD Candidate and Suresh C. Sikka, PhD, HCLD

8:10 AM 0:50 Key Note Speaker: Dolores J. Lamb, PhD, HCLD
"The Genetic and Genomic Basis of Human Male Reproductive Defects"

9:00 AM 0:10 Q&A

Oral Presentations: Abstracts S5A16-S5A20

9:10 AM 0:10 S5A16 Elizabeth Kalmanek, BS - Caspase signaling in ED patients and animal models

9:20 AM 0:10 S5A17 Michael R. Odom, PhD Candidate - Testosterone Replacement Enhances Internal Pudendal Artery Relaxation to Reverse Erectile Dysfunction in a Rat Model of Androgen Deprivation Therapy

9:30 AM 0:10 S5A18 Ryan W. Dobbs, MD, Urology Resident - Sonic hedgehog regulation of cavernous nerve regeneration and neurite formation in aged pelvic plexus

9:40 AM 0:10 S5A19 Lisa A. Baker, PhD Candidate - Targeted depletion of the microtubule regulatory protein fidgetin-like 2 (FL2) enhances axon regeneration and improves erectile function outcomes after cavernous nerve injury in a rodent model of radical prostatectomy

9:50 AM 0:10 S5A20 Andrew T. Gabrielson, Medical Student - Total Testosterone Level Correlates with Intraprostatic Lymphocyte Density

10:00 AM 0:10 Q&A

10:10 AM 0:15 Break
Program in Detail
December 2, 2018

Session 6: Physiology and Pharmacology

Moderators: Ekta Tiwari, PhD Candidate and Johanna L. Hannan, PhD

10:25 AM 0:50 State of the Art Speaker: Francesco J. Demayo, PhD
"Understanding the Role of Progesterone in Reproductive Tract Biology"

11:15 AM 0:10 Q&A

Oral Presentations: Abstracts S6A21-S6A25

11:25 AM 0:10 S6A21 Elvis K Danso, PhD - Characterization of the Biaxial Biomechanical Properties of Human Post-Menopausal Prolapsed and Non-prolapsed Uterosacral Ligament

11:35 AM 0:10 S6A22 Vivian Cristofaro, PhD - Hyperglycemia Induces Detrusor Hyperactivity by Altering the Caveolae-Dependent Regulation of ROCK Signaling Pathway

11:45 AM 0:10 S6A23 Fei Ma, PhD - Central Central (Spinal) Mechanisms of Persistent Bladder Pain: A role for MIF and HMGB1

11:55 AM 0:10 S6A24 Arezoo Geramipour, PhD Candidate - Age-Related Deficits in Sensory-Mediated Reflex Bladder Control in Rat

12:05 PM 0:10 S6A25 Karen I. Hannigan, PhD - Modulation of intracellular Ca2+ in interstitial cells of Cajal and smooth muscle cells by nitrergic neuromuscular transmission in the murine internal anal sphincter

12:15 PM 0:10 Q&A

12:25 PM 1:00 Lunch

Session 7: Novel Therapeutics / Diagnostics

Moderators: Gabrielle L. Clark, PhD Student and Kelvin P. Davies, PhD

Oral Presentations: Abstracts S7A26-S7A30

1:25 PM 0:10 S7A26 Anna S. Nagle, PhD - Measurement of non-voiding rhythmic bladder contractions by M-mode ultrasound during urodynamics in humans

1:35 PM 0:10 S7A27 Maria K Jantz, PhD Student - Selectively activating lower urinary nerves with epidural spinal cord stimulation

1:45 PM 0:10 S7A28 Chaitanya Gopinath, PhD - Microstimulation of sacral dorsal root ganglia enables selective recruitment of sensory afferents of the lower urinary tract

1:55 PM 0:10 S7A29 Robert A. Karam, PhD - Dynamic Time Warping Parameter Optimization for Bladder Event Detection Algorithm

2:05 PM 0:10 S7A30 Nicholas Dias, PhD Student - Aging related alterations of motor unit firing rate in the bulbospongiosus muscle using high density surface electromyography

2:15 PM 0:10 Q&A

2:25 PM 0:15 Break
Program in Detail
December 2, 2018

Awards and Closing Remarks
2:40 PM      0:20    Awards: Matthew O. Fraser, PhD and Sean M. Ward, PhD
3:00 PM      0:15    Closing Remarks: Matthew O. Fraser, PhD

3:15 PM      0:00    Meeting Adjourns

The Top Ranked Abstracts by the Abstract Reviewers:

S1A5 - Bradley A. Potts MD et al. "Striking Differences in the Effects of 83-Adrenoceptor Agonists and Anti muscarinics on Bladder Filling/Voiding Function in Chronic Spinal Cord Injured Rats"

S2A8 - Rosalyn Adam PhD et al. "Neuropilin 2: a novel regulator of gut motility"

S5A16 - Elizabeth Kalmanek BS et al. "Caspase signaling in ED patients and animal models"

S6A22 - Vivian Cristofaro PhD et al. "Hyperglycemia Induces Detrusor Hyperactivity by Altering the Caveolae-Dependent Regulation of ROCK Signaling Pathway"

S6A23 - Fei Ma PhD et al. "Central (Spinal) Mechanisms of Persistent Bladder Pain: A role for MIF and HMGB1"

S7A26 - Anna S. Nagle PhD et al. "Measurement of non-voiding rhythmic bladder contractions by M-mode ultrasound during urodynamics in humans"
Keynote Speaker

Dr. Kim D. Anderson-Erisman, PhD
Department of Physical Medicine and Rehabilitation
MetroHealth Medical Center and Case Western Reserve University (CWRU) School of Medicine
Cleveland, Ohio, USA

Dr. Anderson-Erisman is a Professor in the Department of Physical Medicine and Rehabilitation at the MetroHealth Medical Center and Case Western Reserve University (CWRU) School of Medicine. Her research has focused on translational investigations and bridging the gap between basic science, clinical science, and the public community living with spinal cord injury (SCI). Her training spans the spectrum of SCI research, from cellular and molecular studies, to whole animal and behavioral studies, to human clinical research. Several of her studies have focused on obtaining the perspective of people living with SCI on various aspects of research, including functional priorities, acceptable benefits and risks, preferences for neuroprosthetics, and exercise participation. She has expertise in SCI outcome measures and has conducted a multi-center clinical study evaluating the reliability and validity of the Spinal Cord Independence Measure in the US healthcare setting. In addition to pursuing her own research regarding chronic injury, she was part of the leadership team running the 6 FDA-regulated Schwann cell transplantation clinical trials while a faculty member at the University of Miami. At MetroHealth-CWRU she is continuing her involvement in clinical trials with the team pursuing implanted stimulation devices for SCI and further developing her independent research efforts addressing issues important to people living with SCI with an emphasis on translational research to deploy treatments to the clinic.

Dr. Anderson-Erisman also founded the North American Spinal Cord Injury Consortium (NASCIC) and is the current President. NASCIC brings together like-minded organizations, individuals, and groups to improve research, care, cure, and policies impacting people living with spinal cord injury, their families, and community.

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“Pelvic Dysfunction after SCI: Data from the Lived Experience”

During her presentation, Dr. Anderson-Erisman will present data gathered from people living with spinal cord injury. Regaining bladder, bowel, and sexual function are consistently high priorities across the community. In humans, autonomic dysreflexia appears to be negatively influencing sexual activity. Data suggest the development of neuroplasticity and/or adaptative strategies regarding sexual stimulation and arousal in people living with complete SCI. It appears that improving sensation below the level of lesion may influence multiple components of sexual function. It also appears that returning voluntary control of bladder or bowel function will also influence multiple components of sexual function. Recent data will be presented regarding the community’s interest in neuromodulation strategies to enhance bladder and bowel function.
**State of the Art Speaker**

**International Continence Society Lecturer**

**Dr. Karen D. McCloskey, PhD**
Centre for Cancer Research and Cell Biology, School of Medicine, Dentistry and Biomedical Sciences
Queen’s University Belfast, Belfast, Northern Ireland, UK

Dr Karen D. McCloskey, PhD obtained her PhD in Physiology in 1997 from Queen’s University Belfast, having worked under the supervision of Dr Keith Thornbury on the topic of membrane currents in smooth muscle cells from the urinary tract. Following this, she worked for Professor Noel McHale as a postdoctoral researcher on a project on lymphatic vessels and took a short study visit to University of Nevada, Reno. Appointment as a Senior Research Fellow and Biological Applications Manager at the Centre for Biophotonics, University of Strathclyde, Glasgow, Scotland enabled her to develop independence and she was awarded a New Investigator Grant from the BBSRC to study interstitial cells in the urinary bladder.

Successful application to the Wellcome Trust for a University Award (5-year fellowship, 2003-2008) enabled her to return to Queen’s University Belfast as a Senior Lecturer and open a new laboratory, focused on bladder cell physiology. Collaborations with partners in the European Union and an EU Framework 7 Consortium grant (2008-2012) underpinned new projects and opportunities. Sustained funding from the Royal Society, Action Medical Research, BBSRC and the MRC in addition to medical charities has supported her laboratory and importantly, the training of PhD students, Masters students, undergraduates, technicians and postdocs.

Their current research programmes encompass bladder physiology and we are particularly interested in how radiation therapy impacts the bladder. They also have projects on ion channels in urothelial and prostate cancers and work with clinical colleagues to advance translational research.

She is committed to working with young people from local schools to engage them in physiology and research and host visits to Queen’s University in addition to taking classes in Schools. Her group is passionate about engagement with the public and take part in Open Days and public charitable events e.g. ‘Movember’ activities. She has been Director of the Gender Equality Office in the medical school since 2012 and has led 2 successful Athena SWAN Award applications, recognizing the school’s commitment to advancing and supporting the career progression of academic women.

“Emerging concepts in bladder physiology – new targets for translation?”

The urinary bladder has the dual function of urine storage and emptying. In normal physiology, urine storage represents the state of the bladder for the majority of time, with periodic emptying occurring under voluntary control when socially convenient. The bladder’s ability to store increasingly larger volumes of urine at low intravesical pressures, i.e. compliance, and then efficiently empty from a range of volumes is achieved through the cells of the bladder wall acting as sensors and responders, including the urothelium, sensory/motor nerves, interstitial cells and smooth muscle.

This narrative is underpinned by complex molecular signaling within and between the cellular elements of the bladder wall, which have been the focus of research for many years. Our knowledge of such mechanisms is ever increasing and there is now a need both to integrate information and progress our understanding of how the bladder works in health and fails in disease. Ultimately, our goal as researchers is to improve the health and quality of life of patients with bladder dysfunction; the potential of some of the emerging cellular and molecular targets to be translated for patient benefit will be discussed.
State of the Art Speaker

Dr. Tim Bruns, PhD
Department of Biomedical Engineering
University of Michigan, Ann Arbor, Michigan, USA

Dr. Tim Bruns, PhD is an Assistant Professor of Biomedical Engineering at the University of Michigan. He leads the Peripheral Neural Engineering and Urodynamics Lab, which develops interfaces with the peripheral nervous system to restore autonomic organ function and examine systems-level neurophysiology. His lab has a specific focus on developing new treatments for pelvic organ dysfunction. Dr. Bruns received his PhD in Biomedical Engineering from Case Western Reserve University and completed a postdoctoral fellowship at the University of Pittsburgh

"Neuromodulation for bladder and female sexual dysfunction: tapping into sensory pathways"

Electrical stimulation of nerves, also known as neuromodulation, to control end-organ function has been studied for decades. Successful clinical therapies include applications in hearing, vision, movement disorders, and chronic pain, as well as pelvic organ function. In general, neuromodulation, particularly with implanted pacemaker-like stimulators, is a tertiary line of treatment after more conservative approaches such as lifestyle changes and medications have failed. In recent years, there has been a significant rise in attention for neuro-modulation for organ and autonomic system control, thanks in large part to special funding initiatives from organizations like the NIH, DARPA, and GSK. This era of bioelectronic medicine holds new promises for pelvic organ dysfunctions. In this talk I will review the use of neuromodulation to improve pelvic organ function, specifically highlighting bladder and female sexual function areas that my lab studies, while also discussing other applications.
Dr. Patricia Greenwel, PhD
Division of Digestive Diseases and Nutrition
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Bethesda, Maryland, USA

Dr. Patricia Greenwel, PhD is a Program Director in the Division of Digestive Diseases and Nutrition at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) where she manages and supports a portfolio of grants related to research related to stem cell biology and development of the gastrointestinal (GI) system, epithelial cell biology, basic GI neurobiology and GI lymphatics.

Dr. Greenwel’s academic background includes a PhD in Experimental Pathology from Albert Einstein College of Medicine and post-doctoral work at Mount Sinai School of Medicine in the Department of Molecular Biology. Before joining NIH, she held Faculty positions in the Department of Developmental Biology at Mount Sinai School of Medicine where she studied the genesis and progression of connective tissue disorders.

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“Basic and Translational Research Funding Opportunities from NIDDK/DDN”

Her presentation will be focused at summarizing funding opportunities and other resources offered by NIDDK for investigators interested in research related to digestive diseases, with particular emphasis on basic and translational neurogastroenterology, GI development and stem cell biology, and GI motility and functional disorders. Additionally, her presentation will review initiatives run by NIDDK and other NIH institutes relevant to the interests of the Society for Pelvic Research including the SPARC (Stimulating Peripheral Activity to Relieve Conditions) Program and the recently launched HEAL initiative (Helping to End Addiction Long-term).
**Keynote Speaker**

**Dr. Dolores J. Lamb PhD, HCLD**
Department of Urology and the Center for Reproductive Genomics
Weill Cornell Medicine, New York, New York, USA

**Dr. Dolores J. (Dorrie) Lamb, PhD** joined Weill Cornell Medicine on March 1, 2018 as Vice Chair for Research in the Department of Urology and Director of the Center for Reproductive Genomics. She will hold the Dow Professorship of Urology. She maintains an active presence in both the academic and research communities at Weill Cornell Medicine.

Dr. Lamb is an investigator in the fields of urology, male infertility, steroid hormone action, prostate cancer and genitourinary birth defects. Her experience is unique as she has an extensive background in both the clinical diagnostic and the basic science arenas in men's health as well as having a remarkable record of achievement in the mentoring and development of clinician-scientists.

Dr. Lamb has been recognized by the American Society of Andrology with the Distinguished Andrologist Award as well as by the American Urological Association for significant contributions in the field of reproductive urology and service to the Society for the Study of Male Reproduction. She was also the Ramon Gutierras Lecturer at the AUA’s annual meeting for her lifetime contributions to the field of Urology.

Among her many other distinctions, she received the Distinguished Mentor Award from the American Urologic Association Foundation. She is a former president of both the American Society for Reproductive Medicine and the Society for Male Reproduction and Urology. She was the inaugural recipient of the Distinguished Researcher Award from ASRM as well.

She is a highly recognized NIH-funded researcher whose areas of investigation have focused on the genetics of male infertility, the genomics of genitourinary birth defects, steroid regulated growth of male reproductive tumors, and other areas of benign urologic research.

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"The Genetic and Genomic Basis of Human Male Reproductive Defects"

Urogenital (UG) birth defects comprise some of the most common, yet least studied congenital malformations. Upper tract defects have high morbidity and mortality rates and account for up to 30% of structural birth defects. Mapping of copy number variations(CNVs) revealed that 16p11.2 is a hotspot for UG development. The only gene covered collectively by all the mapped CNVs in patients with UG defects was MYC-associated zinc finger (MAZ). MAZ encodes a transcription factor with a similar consensus sequence to that of WT1. Maz is ubiquitously expressed, and a Crispr-Cas9 mouse model of Maz loss of function results in perinatal lethality. Survival rates are dependent on Maz copy number. Homozygous loss of Maz results in high penetrance of CAKUT, and Maz is haploinsufficient for normal bladder development. MAZ, once thought to be a simple housekeeping gene, encodes a dosage sensitive transcription factor that regulates urogenital development and contributes to the congenital malformations of the 16p11.2 phenotype. Our studies revealed the presence of other CNVs present in UG patients. Dosage-sensitive genes identified within these CNVs by our group (VAMP7, CRKL, OTX1, KTCD13, ZEB2, E2F1, MECP2) caused key signaling pathways to be altered in fascinating ways never considered. These gene dosage changes resulted in anomalous UG development in mouse models recapitulating the human phenotype.
State of the Art Speaker

Dr. Francesco J. Demayo, PhD

Reproductive and Developmental Biology Laboratory / Pregnancy and Female Reproduction Group
National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina, USA

Dr. Francesco J. DeMayo, PhD is Senior Principal Investigator and Chief of the Reproduction and Developmental Biology Laboratory. He received his B.S. in General Studies at Cornell University and his M.S. and Ph.D in Physiology at Michigan State University. He continued his postdoctoral training at Baylor College of Medicine where rose to the rank of Cullen-Duncan-McAsahan Endowed Chair in Cancer Research, and Professor of Molecular and Cell Biology and Pediatrics. During his tenure at Baylor College of Medicine he has received the Michael E DeBakey Research Award and the Society for the Study of Reproduction Research Award.

"Understanding the Role of Progesterone in Reproductive Tract Biology"

The ovarian steroid hormone, Progesterone, acting through its cognate receptor, the Progesterone receptor, Pgr, is critical for regulating all processes of the ability of the uterus to support embryo implantation and pregnancy. The Pgr consist of two isoforms, the PgrA and the PgrB. The Pgr isoforms act in all compartments of the uterus. In the preimplantation period the Pgr expressed in the uterine epithelium is critical for enabling the uterine epithelium to become receptive and priming the stroma cells to undergo the decidual reaction to support the implanting embryo. In the stroma compartment the Pgr is necessary for the progression and maintenance of decidual response. Finally in the myometrium the Pgr is critical for maintaining the uterine smooth muscles in a state that will allow the growth of the developing fetus. Utilizing genetically engineered mice in combination with transcriptomic and cistromic analysis we have begun to map the interactions of Pgr with other transcription factors and signaling pathways in the regulation of uterine receptivity, and the ability of the myometrium to support pregnancy. This technology has also been used to investigate the role of PgrA and PgrB in the mouse uterus by specific ablation and overexpression of these isoforms. The pathways identified in the mouse have been validated in primary human samples, primary human endometrial culture cells and human clinical databases to validate their clinical significance. Currently, with the explosion of genome editing tools such as CRISPR/Cas9 the ability to dissect the molecular pathways regulating uterine receptivity will be made simpler and the ability to investigate multiple genetic alterations at once will facilitate the understanding of how the uterus is able to support pregnancy.

This work was supported by the NIEHS, and grants from the NICHD, March of Dimes and Burroughs Welcome Fund.
Leading Continence Research and Education

Call for Abstracts: 1 March - 1 April 2019

International Continence Society 48th Annual Meeting

www.ics.org/2019
Title: Assessment of bladder function after year-long lower spinal root transection and surgical reinnervation using behavioral measures

Authors: Danielle M. Salvadeo¹, Ekta Tiwari², Nagat Frara³, Lucas Hobson¹, Alan S. Braverman¹, Mary F. Barbe¹, Michael R. Ruggieri, Sr.¹,²,³

Affiliations: ¹Departments of Anatomy and Cell Biology, Lewis Katz School of Medicine, ²Electrical and Computer Engineering, College of Engineering, Temple University, and ³Shriners Hospitals for Children, Philadelphia, PA, USA.

Introduction/Objectives: We aim to surgically reinnervate a lower motor neuron neurogenic bladder via obturator-to-pelvic and sciatic-to-pudendal nerve transfer. Behavioral observations provide the most global assessment of functional recovery. Our aim here was to determine whether observed micturition postures disappear in decentralized animals and reappear after reinnervation.

Methods: Three groups of female canines were used: 1) Long-term survival (12 months) after transection of all ventral and dorsal sacral roots, the dorsal roots of L7 and hypogastric nerves, bilaterally (n=4); 2) Procedures described in Group 1, followed by obturator-to-pelvic nerve and sciatic-to-pudendal nerve transfer (n=3); 3) Unoperated control animals (n=3). Urination postures were monitored for 24 hours at monthly intervals using video cameras over the housing cages. Bacteriuria was tracked midway through the study with urine Multistix® test strip assays, body temperature measurements, and cultured urine specimens by a commercial clinical microbiology laboratory. Biweekly awake cystometry assessed awareness of a bladder fullness and ability to empty.

Results: Three of four animals in Group 1 showed micturition postures up to 12 months postoperatively, with the fourth animal exhibiting an intermediate posture unlike the typical micturition posture. While the occurrence of bacteriuria for three of the animals is unknown due to lack of testing, we noted that bacteriuria in one animal coincided with the observed postures. We similarly observed an increase in micturition postures with the presence of bacteriuria in two of three animals in Group 2, prior to reinnervation. These postures completely disappeared in all animals with successful antibiotic clearance of the bacteriuria. Filling cystometrograms were not performed in animals with active bacteriuria. All of the Group 3 control animals showed micturition postures and bladder emptying within 3 minutes after removal of the Foley catheter and recovered voided volume was 75-79% of their cystometric bladder capacity. During the awake cystometry, none of the animals in Group 1 appeared distressed by bladder filling. In contrast, one of three animals in Group 2 tested at 6 and 7 months after nerve reinnervation procedures made vocalizations that suggested discomfort, and therefore sensation, after filling.

Conclusions: Although we predicted a global loss of sensation after transecting the sacral roots and dorsal roots of L7, we observed frequent micturition postures suggestive sensation, possibly due to concomitant UTIs rather than bladder fullness. Furthermore, observations made during awake cystometry suggest that the nerve transfer reinnervation procedure lead to recovered sensitivity of bladder fullness.

Funding Source(s): NIH: NIH-NINDS NS070267 to MRR and MFB
S1A2

Title: Inflammation in Development of LUTS following Radical Prostatectomy.

Authors: Guillermo A. Villegas¹, Moses T. Tar¹, Yi Wang¹, Kelvin P. Davies¹$ and Sylvia O. Suadicani¹$.

Affiliations: ¹ Department of Urology, Albert Einstein College of Medicine, Bronx, NY, USA. $Senior co-authors.

Introduction/Objectives: Radical prostatectomy (RP) is a commonly used treatment option for localized prostate cancer and carries a high risk for development of Lower Urinary Tract Symptoms (LUTS) which is highly detrimental to the post-surgical well-being of men. Although the ability to recover continence after RP has improved with use of robotically assisted RP (RARP), prevalence of incontinence is still considerable. In this regard, even for RARP, surgical trauma still occurs and needs to be considered as a factor contributing to post-surgical urological dysfunction. Inflammation has been suggested to be a major factor in the development of urogenital dysfunction after RP, although at present there are limited preclinical studies validating this association. The objective of the present study was thus to determine if even after nerve sparing RP (NSRP), the associated surgical trauma triggers inflammatory responses in the bladder that are accompanied by post-surgical LUTS.

Methods: Male Sprague Dawley Rats were divided into 3 groups (N=3-6) and subjected to the following surgical procedures: Naïve (no surgery); laparotomy only (LP) and LP with cavernous nerve detachment from the prostate (NSRP). Bladder function was assessed using the void stain on paper (VSOP) method before and 3 days after the surgeries. VSOP was performed for 8-hours on filter paper sheets placed on the bottom of the cages and urine stained spots examined under UV light at 302nm. Voiding frequency was calculated from the number of voided spots during the 8-hours assessment. Animals were sacrificed 3 days-post-surgery and bladders were collected; total RNA was isolated from the mucosa and detrusor tissues. Gene expression of inflammatory markers was assessed by RT-qPCR. Results were analyzed by paired and unpaired t-test.

Results: Rats that underwent NSRP developed significant LUTS as denoted by a significant decrease in spontaneous voided urine volumes and increase micturition frequency at 3-days post-surgery. Analyses of the mRNA expression showed significant increase in expression of TNFα, IL-1β, IL-6, NGF, Nf-kb and a dramatic decrease of HO-1 in NSRP group compared with laparotomy and naïve animals, particularly in the bladder mucosa.

Conclusions: Local inflammatory responses occur in the bladder following NSRP and are accompanied by LUTS. These findings support the proposed role of inflammation in development of LUTS following RP and provide pre-clinical evidence for the use of anti-inflammatory strategies to improve post-surgical RP outcomes.

Funding Source(s): Support was provided from NIH R01 DK109314 and NY State SCIIRB C31611GG
S1A3

Title: BDNF is Essential for Pudendal Nerve Motor Branch Functional Recovery

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Introduction/Objectives: Stress Urinary Incontinence (SUI) is the leakage of urine due to an increase in abdominal pressure effecting 30% of women over the age of 40; its primary risk factor is childbirth. As the baby’s head passes through the birth canal it injures the pudendal nerve (PN), which innervates the external urethral sphincter that provides urethral resistance and maintains continence. Increased motor latency in incontinent women after childbirth suggests that PN function and recovery are important to the multifactorial continence mechanism. A PN crush (PNC) model of SUI demonstrates that BDNF is upregulated after PNC, suggesting that it is necessary for PN regeneration. We hypothesized that BDNF is essential to PN functional recovery following PNC.

Methods: In this experiment we reduced active BDNF by administering its receptor, TrkB, to block the action of BDNF and determine if this reduces recovery from PNC. Sprague-Dawley rats (15) received sham bilateral PNC with implanted osmotic pumps (Alzet model 2002) containing saline (SPNC + S). Additionally, 30 rats received bilateral PNC with osmotic pumps, containing saline (PNC + S) or Fc-TrkB chimera (PNC + TrkB). Three weeks later the animals underwent functional testing consisting of leak point pressure (LPP) with simultaneous PN motor branch potential (PNMBP) recording followed by PN sensory branch potential recording during clitoral brushing (PNSBP). One-way ANOVA on Ranks followed by a Dunn’s test was used to indicate significant differences (p<0.05).

Results: PNC + TrkB PNMBP firing rate was significantly decreased compared to PNC + S, while PNC + S was not significantly different from SPNC + S. PNSBP firing rate & amplitude was not significantly different between any of the groups. LPP was not significantly different between the groups.

Conclusions: BDNF is essential to PN motor branch regeneration after PNC, however BDNF may be necessary but not essential for PN sensory branch regeneration. Continence is maintained by multiple factors, explaining why a decrease in PN motor function did not lead to a decrease in LPP, a comprehensive measure of continence.

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Title: Neuroprotection of sphincter motoneurons after spinal cord injury

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Introduction/Objectives: Spinal cord injury (SCI) results in large necrotizing lesions that destroy tissue and disrupt spinal tracts. We have previously demonstrated that after SCI, surviving somatic motoneurons undergo dramatic dendritic atrophy with concomitant behavioral deficits. Treatment with androgens or estrogens has protective effects on multiple sequelae of SCI, especially in protecting somatic motoneurons and the muscles they innervate from SCI-induced atrophy. Deficits in autonomic function are also common after SCI, with urinary problems being the most common quality of life problems reported by SCI patients. In this experiment, we hypothesized that after a clinically relevant contusive SCI model, similar protective effects of gonadal hormones would be present in sphincter motoneuron structure and function.

Methods: Gonadally intact young male rats received either a sham or a T9 contusion injury (10 g, 25 mm) using an NYU impactor. Immediately following contusion, rats were implanted with subcutaneous Silastic capsules filled with estradiol (E) and dihydrotestosterone (DHT) or left blank. Micturition assessment (void frequency and volume) was performed at 3 weeks after SCI. Four weeks after SCI, motoneurons innervating the external urethral sphincter (EUS) muscle were labeled with cholera toxin-conjugated HRP, and dendritic arbors were reconstructed in three dimensions; lesion volume, and tissue sparing were also assessed.

Results: Void frequency decreased and void volume increased after SCI; both were dramatically improved by treatment with E+DHT. Contusion injury resulted in large spinal cord lesions, and treatment with E+DHT had no effect on lesion size, percent total volumes of lesion, or spared white and gray matter. Similar to what we have previously reported for somatic motoneurons, we expect that SCI will result in decreases in EUS motoneuron dendritic length; treatment with E+DHT is expected to prevent SCI-induced atrophy in EUS motoneurons, providing a mechanism for the protective effects on micturition.

Conclusions: Voiding requires the integration of autonomic and somatic pathways within the lumbosacral cord. SCI-induced bladder areflexia recovers through a slow re-emergence of involuntary reflex micturition mediated by these spinal reflex pathways. Treatment with hormones resulted in improvement in both void frequency and volume after SCI, resulting in micturition patterns that closely approximated the normal pattern. Because the improvements in micturition were seen in groups with lesions comparable to those of untreated animals (and thus with similar disruptions in descending tracts), it is possible that hormones could be acting directly on the motoneurons or locally on the autonomic and somatic pathways within the lumbosacral cord, indirectly supporting EUS motoneuron morphology and function. Together, these results indicate that the use of gonadal hormones could be an effective treatment after SCI.

Funding Source(s): Indiana University/Purdue University, Signature Center Initiative – Center for Spinal Cord and Brain Injury Research.
Title: Striking Differences in the Effects of β3-Adrenoceptor Agonists and Antimuscarinics on Bladder Filling/Voiding Function in Chronic Spinal Cord Injured Rats

Authors: Bradley A. Potts¹, Danielle J. Degoski², Jillene M. Brooks², Matthew O. Fraser¹,²,³

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Introduction/Objectives: β3-adrenoceptor agonists (BARA) and antimuscarinics are mainstays in the treatment of overactive bladder. We previously demonstrated significant positive effects with a rat-specific BARA, CL-316,243 (CL), in chronic suprasacral spinal cord transected (SCI) rats. We present the results of a selective post-hoc analysis of CL and atropine effects from a multidrug study designed to assess myogenic vs neurogenic contributions to SCI-induced neurogenic detrusor overactivity (NDO).

Methods: External urethral sphincter (EUS) EMG electrodes and catheters (femoral vein, ureteral diversion and transvesical) were placed in isoflurane-anesthetized female Sprague-Dawley rats (4 weeks post-SCI at T9-10, n=14). Conscious continuous cystometry was performed for ≥60min using Ballman cages. The infusion was stopped, bladder emptied, and vehicle (normal saline) was administered prior to resuming bladder infusion. Following 30min, the infusion was again stopped, the bladder emptied, and i.v. drugs were delivered prior to subsequent 30min infusion cycles. Six animals received 0.4mg/kg atropine and the other eight received 10mg/kg verapamil; following the same methods, the verapamil-treated group received 100μg/kg CL prior to the next fill cycle. Total bladder capacity (TBC), filling compliance, non-voiding contraction (NVC) counts, frequency and average amplitude, voiding duration (VD) and efficiency (VE), and EUS phasic firing frequency (PFF) were measured/determined. Data were analyzed using non-parametric 1- or 2-Way RM-ANOVA, or linear regression.

Results: VP had no effect on any parameter and, due to its 2min half-life, we compared the effects of atropine to CL. Both atropine and CL significantly increased TBC (47%, P=0.0016 and 61%, P=0.0018, respectively), but atropine increased NVC counts and frequency (167%, P=0.0001 and 31%, P<0.0001, respectively) and CL decreased NVC frequency (-22%, P=0.0009). Both drugs decreased overall NVC amplitudes (P=0.0221). Atropine significantly decreased VE (-68%, P=0.0060) and VD (-42%, P=0.0009), and, in these animals, VD and VE were positively associated (P=0.0007, R2=0.70). Compliance and PFF were unaffected by any drug treatment.

Conclusions: While TBC was increased and NVC amplitude decreased by both CL and atropine, atropine actually increased NVC count and frequency, while CL reduced NVC frequency. CL did not affect VE, and the strong relationship between VD and VE in atropine treated animals suggests that VD is also an important factor for VE effects of antimuscarinics. These results support the continued study of BARA for NDO treatment.

Funding Source(s): DoD SCIRP IIR–SC110031
Title: Calcium waves and spatio-temporal mapping reveals myosalpinx excitability and propagation along the murine oviduct

Authors: Sean M. Ward¹, Jasmin Wächter¹, Ha young Jo¹, Bo Hyun Kim¹, Ju Hyeong Lyu¹, Sung Jin Hwang¹ & Yulia Bayguinov¹

Affiliations: ¹Department of Physiology and Cell Biology, University of Nevada, Reno School of Medicine, Reno, NV 89557

Introduction/Objectives: Oviducts, or fallopian tubes (in primates), are smooth muscle-lined tubular organs that facilitate several essential physiological processes including gamete transport, ovum fertilization and early embryo development. Contractions of the oviduct smooth muscle (myosalpinx) and the wafting motion of the ciliated epithelium that lines these tubes, facilitate bi-directional transport of gametes so that the newly released ovum(s) is transported in one direction (pro-uterus) while spermatozoa are transported in the opposite direction (pro-ovary). For successful fertilization to occur, these transport processes must be temporally coordinated so that the ovum and spermatozoa meet each other in the ampulla; the site of fertilization. Once the ovum is fertilized, the early embryo then begins another precisely timed journey toward the uterus for implantation. Myosalpinx contractions also facilitate this journey while luminal secretions from secretory epithelial cells aid early embryo maturation and influence gamete viability. In order to develop a better understanding of the excitable nature of the oviduct myosalpinx, we utilized a novel mouse model with a genetically engineered calcium indicator (PDGFRα/cre-Gcamp6f/loxP) that was expressed in the myosalpinx. Specific questions to be addressed were: (i) are all regions of the oviduct excitable? (ii) how does propagation occur along the different segments of the oviduct? (iii) did activity that originated in the oviduct propagate to the adjacent uterus? (iv) what were the cellular mechanisms responsible for the generation and propagation of calcium waves?

Methods: Calcium imaging and spatiotemporal mapping was performed at different sites along the oviduct. Simultaneous intracellular microelectrode recordings and calcium imaging were also performed. Confocal microscopy was used to determine cellular expression of Gcamp6.

Results: PDGFRα/cre-Gcamp6f/loxP mice displayed robust spontaneous calcium waves throughout all regions of the oviduct. Calcium waves propagated from ovary to uterus, but also from uterus to oviduct, often colliding in a specialized region within the Ampulla/isthmus. Calcium waves that originated in the isthmus often propagated into the uterus activating an excitable wavefront that spread along this organ. Electrical slow waves were responsible for the generation of calcium waves. Calcium waves were sensitive to removal of extracellular calcium, inhibiting intracellular calcium stores with cyclopiazonic acid (CPA) and were reduced or blocked by an Ano1 calcium-activated chloride channel (CaCC) inhibitor. Caffeine also inhibited oviduct calcium waves.

Conclusions: For the first time, we are able to track the generation and propagation of calcium waves as an indicator of oviduct myosalpinx excitability. Propagation of calcium waves in both directions along the oviduct suggests that myosalpinx activity contributes to both gamete transport. These events relied on the release of intracellular calcium and were likely generated by the CaCC Ano1.

Funding Sources: Supported by NIH DK57236 & DK41315.
Title: Transcriptomic analysis of bladder PDGFRα⁺ cells in early diabetic bladder

Authors: Tong Zhou, Haeyeong Lee, Kenton M. Sanders, Sang Don Koh

Affiliations: Tong Zhou, Haeyeong Lee, Kenton M. Sanders, Sang Don Koh

Introduction/Objectives: Early DBD is associated with an increase in NVCs and a decrease in compliance. We have reported that PDGFRα⁺ cells contribute to the stabilization of membrane excitability during filling. When excitability-related transcripts are suppressed in PDGFRα⁺ cells, detrusor muscles display increased NVCs and TCs, and submucosal fibrosis decreases the compliance in the early stages of DM. Thus, changes in transcriptomic profile of PDGFRα⁺ cells could result in symptoms of detrusor overactivity (DO) in early DM.

Methods: Akita mice (8wks and 16 wks old) were used for RNAseq analysis in submucosa and detrusor layers. The submucosal layer was dissected free of detrusor. RNA quality was verified in the Nevada Genomics Center and mRNA was collected and analyzed by RNA-seq (BGISEQ-500, Cambridge, MA, USA). Using RNA-seq, we profiled the transcriptomes of detrusor and submucosa from control and Akita mice (8 wks). We also used freshly dispersed PDGFRα⁺ cells from PDGFRα/eGFP mice. These cells were sorted and purified by FACS. PDGFRα⁺ cells from submucosa and detrusor were used for RNA-seq.

Results: Gene expression fold change was computed for each gene between control and Akita mice. We observed a significant negative correlation in fold change between detrusor and submucosa cells suggesting differences in Akita-induced gene deregulation in detrusor and submucosa PDGFRα⁺ cells. Pdgfra expression in detrusor and submucosa of control and Akita mice were compared. Pdgfra was significantly upregulated in Akita submucosa compared to Akita detrusor (FDR<5%). These data suggest that submucosal Pdgfra may play a role in bladder fibrosis. ECM-receptor interaction is one of the key pathways associated with fibrotic processes. Therefore, we computed the ECM-receptor interaction pathway score in Akita submucosa by the FAIME algorithm, and the ECM-receptor interaction pathway score was significantly higher in Akita submucosa than in controls (FDR<5%). The KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis of RNA-seq data reveals seven up-regulated KEGG pathways in sorted submucosal PDGFRα⁺ cells regarding the fibrotic process. These results suggest that the genes differentially expressed in submucosal PDGFRα⁺ cells are involved in the fibrotic processes. To further investigate the mechanism(s) of fibrosis in PDGFRα⁺ cells, we identified 27 genes up-regulated in PDGFRα⁺ cells (the genes with FDR<5% and at least 3-fold increased expression in submucosal PDGFRα⁺ cells compared with unsorted cells from our RNA-seq dataset).

Conclusions: These data suggest that transcriptomic deregulation in whole tissues in DM is causatively associated with phenotypic changes in specific cell populations, which might play a role for diabetic bladder dys-function.

Funding Source(s): NIH/NIDDK R01 DK098388
Title: Neuropilin 2: a novel regulator of gut motility

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Introduction/Objectives: Profound changes in gut motility and colonic smooth muscle contraction are observed in inflammatory bowel disease states such as colitis. However, the mechanisms that contribute to altered motility and contractility under these circumstances are incompletely defined. Recent data from our group have identified the smooth muscle of the gastrointestinal tract as a major site of expression of neuropilin 2 (Nrp2) in vivo. Nrp2 and the related molecule neuropilin 1 are 130-140 kDa transmembrane receptors for the class 3 semaphorin (SEMA3) family of axonal guidance regulators and for members of the vascular endothelial growth factor (VEGF) family that regulate angiogenesis. Based on recent data from our group implicating Nrp2 as a novel regulator of bladder smooth muscle contractility, the objective of this study was to determine the functional significance of Nrp2 in gastrointestinal smooth muscle.

Methods: Two mouse models were used to explore a role for Nrp2 in gastrointestinal motility: (i) SM22alpha-Cre; Nrp2 fl/fl mice to enable inducible smooth muscle-specific deletion of Nrp2 in vivo under controlled conditions and (ii) wild type Balb/c mice exposed to dextran sodium sulfate to evoke acute colitis. Gut tissues were subjected to isometric tension testing to characterize contractility. Gut transit was evaluated by charcoal meal assay. Nrp2 levels were assessed by immunohistochemical staining and immunoblot analysis of tissues and of primary colonic smooth muscle cells exposed to inflammatory cytokines.

Results: Smooth muscle-specific deletion of Nrp2 led to an increase in force generation of colonic rings, upon exposure to electrical field stimulation or treatment with the cholinergic agonist, carbachol. Nrp2 deletion also led to elevated gut transit as assessed by charcoal meal assay. In response to colitis, Nrp2 levels were found to decrease in the distal colon, in parallel with elevated contractility of distal colon rings. In addition, treatment of primary human colonic smooth muscle cells with IL-4, representative of the T\textsubscript{H2} inflammatory milieu associated with DSS colitis, decreased Nrp2 levels while treatment with TNF-a, characteristic of the T\textsubscript{H1} response in colitis increased Nrp2 levels.

Conclusions: These observations suggest that dynamic alterations in Nrp2 under conditions of inflammation may underlie altered contractility of intestinal smooth muscle, resulting in dysmotility. Moreover, these findings suggest that Nrp2 may represent a novel therapeutic target for restoration of normal gut motility.

Funding Source(s): Harvard Digestive Diseases Center P&F Grant; NIDDK R01 DK104641; VA BX1790.
Title: Basal and filling-induced degradation of adenosine 5’-triphosphate (ATP) at suburothelium/lamina propria of the murine bladder

Authors: Violeta N. Mutafova-Yambolieva*, Benjamin Kwok, Priya Kukadia

Affiliations: Department of Physiology and Cell Biology, University of Nevada Reno School of Medicine, Reno, NV 89557

Introduction/Objectives: Studies of urothelial cells exposed to cell swelling, hydrostatic pressure changes or drag forces, in bladder sheets subjected to stretch or hydrostatic pressure changes or in lumens of filled bladders, have suggested that ATP released from urothelium into suburothelium/lamina propria (LP) activate afferent nerves and mechanisms controlling detrusor excitability. Once released, ATP is rapidly degraded to ADP, AMP and adenosine (ADO) that might also affect bladder excitability. None of these approaches, however, has enabled direct assessment of availability and metabolism of ATP at suburothelium/LP during filling. The goal of this study, therefore, was to evaluate the degradation of ATP at the anti-luminal aspect of the urothelium at rest and during filling.

Methods: We developed a novel ex vivo mouse bladder preparation, consisting of intact urothelium and LP but with the detrusor smooth muscle removed, that allows direct access to the anti-luminal surface of urothelium during filling. Denuded bladder preparations of C57Bl6/J mice (10-12 weeks of age) were placed in 3-ml chambers and bathed in oxygenated Krebs bicarbonate solution (KBS, 37°C). To evaluate the anti-luminal degradation of ATP, 1Nº-etheno-ATP (εATP, 2 μM) was added to the external solution bathing the LP surface of the preparation. Aliquots of extraluminal fluid were collected before adding the εATP substrate and at 2-10-minute intervals after starting bladder filling with KBS at 15 ml/min. The degradation of εATP was evaluated by measuring the decrease of εATP and the increase of the products εADP, εAMP, and εADO by HPLC-FLD.

Results: We found significantly larger degradation of εATP in bladders that were filled to a typical voiding volume/pressure as opposed to those left unfilled (p<0.05). The difference found between stretched and unstretched bladders can be explained by the activity of a releasable enzyme that was found to hydrolyze ATP in the absence of bladder tissue. There was a significant increase in the amount of ATP hydrolysis by this releasable enzyme when bladders had been filled compared with unfilled preparations (p<0.05), suggesting that the release of this factor is likely stretch-dependent. Interestingly, while this stretch-released enzyme hydrolysεεATP to produce εADP and εAMP, hydrolysis of εAMP to adenosine is unaffected by bladder stretch, indicating little to no releasable AMPase factor.

Conclusions: Our results provide evidence of a stretch-induced releasable ATPase that appears to be necessary in regulating purine distribution at lamina propria. Furthermore, the novel detrusor-denuded bladder preparation might be instrumental for understanding possible asymmetry in availability of biologically active mediators at the luminal and anti-luminal aspects of urothelium during bladder filling.

Funding Source(s): NIH grant DK 41315
**S2A10**

**Title:** A unique population of PDGFRα⁺ cells are expressed in the Cynomolgus monkey internal anal sphincter.

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**Introduction/Objectives:** A second class of interstitial cells have recently been identified in the gastrointestinal (GI) tract. These cells initially described as “fibroblast-like cells” highly express the receptor tyrosine kinase, platelet derived growth factor receptor alpha (PDGFRα) giving rise to the name “PDGFRα⁺ cells”. In studies of the large and small intestine intramuscular PDGFRα⁺ cells have been shown to play an important role in purinergic inhibitory neuromuscular transmission (NMT). Specifically, purines activate P2Y1 receptors on PDGFRα⁺ cells leading to calcium release, activation of calcium activated potassium (SK3) channels and generation of an inhibitory junction potential (IJP) which conducts to adjacent cells. Our studies have demonstrated that purinergic NMT is present in the monkey rectum but absent from the IAS. Thus, we compared the various mediators of purinergic NMT described above to determine the extent to which each is present and functional in these two muscles.

**Methods:** Contractile activity was measured in isolated strips of the *Cynomolgus* monkey IAS and rectum. Expression levels of PDGFRα, SK3 and P2Y receptor genes were evaluated with qPCR. Protein expression of PDGFRα, SK3 and nNOS was examined with immunohistochemistry.

**Results:** The IAS and rectum resembled one another in that: 1) PDGFRα⁺ cells were located in close proximity to nNOS⁺ inhibitory motor neurons, 2) SK3 channel protein was expressed predominately in PDGFRα⁺ cells, 3) the profile for P2Y1 receptor gene expression was the same, and 4) the profile for SK1-3 gene expression was very similar. The IAS and rectum differed in that: 1) PDGFRα⁺ cells were spindle-shaped in the rectum and stellate-shaped in the IAS, 2) contractile activity was continually suppressed by SK channels in rectum but not the IAS, 3) further activation of SK2/3 channels with CyPPA (30 μM) inhibited contraction 5X faster in rectum than in IAS, and, 4) the P2Y1 receptor activator MRS2365 (1 μM) caused contractile inhibition in the rectum but increased contraction in the IAS.

**Conclusions:** The properties of purinergic NMT and PDGFRα⁺ cells in rectum were very similar to those of other intestinal muscles whereas those of the IAS differed. The absence of purinergic NMT in the IAS could not be attributed to the absence of either P2Y1 receptors or SK3 channels. Rather, it appears to be due to poor coupling between SK3 channels on PDGFRα⁺ cells and the established pathways that activate them. Thus PDGFRα⁺ cells in the IAS appear to be functionally unique. Learning more about these unique cells in the IAS may aid in developing novel strategies for the prevention and/or treatment of defecatory disorders in humans.

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S3A11

Title: Improved electrophysiological techniques to monitor hypogastric nerve activity during bladder filling in canines.

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Introduction/Objectives: This paper reports refined electrophysiological methods for monitoring hypogastric nerve activity during bladder filling in intact and acutely lumbosacral-decentralized bladders.

Methods: The effects of electrical stimulation of hypogastric nerves or lumbar roots on detrusor pressure were determined. The effects of isoflurane versus propofol anesthetics on hypogastric nerve stimulation evoked detrusor pressure were compared. Hypogastric nerve activity was recorded using custom-made bipolar electrodes during bladder filling before and after nerve transection proximal to the recording electrode to eliminate efferent nerve signals. Recordings were performed using a low noise amplifier at a gain of 10 k, sampled at 20 kHz, and band-pass filtered between 500 Hz and 3 kHz.

Results: Electrical stimulation of hypogastric nerves evoked low amplitude detrusor pressure in both intact and decentralized bladders that was not different between two anesthetics. Upper lumbar (L2) ventral root stimulation evoked detrusor pressures were suppressed but not statistically significantly eliminated after transection of hypogastric nerves and all spinal roots below L5. Recordings showed a decreased amplitude of hypogastric nerve activity as the bladder reached maximum capacity in intact and decentralized bladders, but no change with filling when recording only afferent activity in intact bladders.

Conclusions: Based on hypogastric nerve and lumbo-sacral cord/roots stimulation results pre and post hypogastric transection, it can be concluded that a more complete decentralized canine bladder model requires transection of the lumbosacral spinal roots innervating the bladder as well as the hypogastric nerve prior to reinnervating the bladder with nerve transfer surgeries. We also found the functional motor effects of upper lumbar ventral horn projections to the bladder that may not be physiologically significant for bladder emptying. Based on our results from hypogastric nerve recording in normal intact and acutely decentralized bladders, this can be interpreted that our refined electroneurogram recording methods may be suitable for evaluation of the effectiveness of nerve transfer strategies for bladder reinnervation by monitoring sensory and motor activity in the transferred nerves.

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Title: Wireless Real-Time Sensor Platform for Monitoring Bowel State and Function

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Introduction/Objectives: The neural control of bowel function during content mobility, storage, and defecation is not well-understood. A more complete understanding may be possible with electrophysiology or pharmacology studies that include functional measures of the bowel in conscious subjects. There are few options for continuous bowel monitoring in awake subjects. Therefore, we are developing a wireless sensor platform as a research tool for measuring bowel pressure, volume, and content for use in experiments of bowel function.

Methods: The bowel sensor was based on a core wireless sensor platform and its form factor was designed for multi-day monitoring in the porcine colon. The prototype is approximately 40 mm long and 7 mm wide, and it can be affixed to the bowel mucosa using an endoscopic hemoclip. The initial sensor prototype comprised a miniaturized circuit board with three sensor modalities, including pressure, conductivity, and capacitive sensors. Volume is approximated from conductivity and conductance measurements. Initial prototype sensor devices were coated in biocompatible epoxy and connected to a computer for data collection. Accuracy and precision of sensors were first characterized through benchtop testing in a colon phantom. The functionality of the sensors was further evaluated in 3 preliminary acute in vivo experiments. The device was first inserted into the rectum through the anus and stool was moved across the device. Then, after performing a laparotomy, the colon was incised and the sensor device was inserted. The device was tested (1) in an empty colon and (2) in a colon filled with stool. Standard pressure manometry catheters provided control pressure data.

Results: Benchtop results showed that the pressure sensor was capable of detecting controlled pressure changes with a RMSE of 3.7 cmH2O. Regression analysis for the conductivity measurements showed a maximum error of 19% and a RMSE of 675 μS/cm. For volume calculation, there was a maximum error of 26% and a RMSE of 4.5 mm for the diameter of a stool. In vivo conductivity measurements were sensitive to stool movements. When the device was covered with stool, adding more stool created further shifts in the conductivity measurement. The conductivity method was able to distinguish between a full or empty bowel. The pressure sensor recorded changes in bowel pressure in response to abdominal compressions and slow-wave bowel contractions.

Conclusions: We have developed a sensor platform for real-time monitoring of bowel state and activity. The pressure sensor measured evoked and spontaneous colon activity in vivo in correlation with standard pressure manometry catheters. Initial results from in vitro and preliminary in vivo studies suggest that volume estimation from conductivity measurements is feasible. Further work is needed to refine and validate the sensor device in vivo. This device has the potential to provide data in awake, behaving subjects in chronic experiments of bowel function.

Funding Source(s): Research was funded by the NIH SPARC program, NIH OT2OD023873.
Title: Progerin causes disruption in enteric neurons and interstitial cells of Cajal in the colons of Imna^{HG-C} mice

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Introduction/Objectives: Hutchinson–Gilford progeria syndrome (HGPS; premature aging syndrome) is caused by a point mutation in exon 11 of LMNA, the gene for lamin C and prelamin A (the precursor to mature lamin A). Lamin A constitutes a major structural component of the lamina, a scaffold of proteins found inside the nuclear membrane of cells. Neurons in the brain produce lamin C but almost no lamin A, a consequence of the removal of prelamin A transcripts by miR-9, a neuron specific microRNA. Progerin is a truncated version of Lamin A protein that is involved in HGPS. In HGPS, aberrant splicing and processing of lamin A produces an internally truncated protein lacking 50 amino acids called progerin, which induces early cellular senescence and is associated with increased DNA-damage signaling. We have previously shown that progerin and full-length farnesyl–prelamin A are toxic to enteric neurons and produces an achalasia-like disorder in the esophagus of the GI tract. We sought to determine whether the expression of Progerin affected the colon of the GI tract. To investigate this, we utilized a new Lmna knock-in allele, Lmna^{HG-C}, which produces progerin transcripts lacking a miR-9 binding site resulting in the robust expression and buildup of toxic Progerin in expressing cells.

Methods: Gross morphological analysis and confocal immunohistochemistry was used to examine the colons of Lmna^{HG-C} mutants and wildtype controls. Intracellular microelectrode recordings were used to examine functional changes in colonic muscles.

Results: Lmna^{HG-C} mutant mice developed greatly distended colons and intestines compared to wildtype controls. Protein Gene Product 9.5 (PGP9.5) was used as a pan-neuronal marker to visualize enteric neurons and nerve fibers within the muscle layers. vAChT and nNOS was used to identify excitatory and inhibitory motor nerves, respectively. There was a marked decrease in all classes of enteric neurons and nerve fibers in the GI tracts of Lmna^{HG-C/+} mice. Interestingly, there was also a significant loss of interstitial cells of Cajal (ICC) in the GI tracts compared to controls. Intracellular recordings and isometric force measurements revealed greatly disrupted spontaneous and neurally evoked motor activity in Lmna^{HG-C} mice. Associated with a loss of enteric nerves and ICC was a huge distension in the colon wall.

Conclusions: These data confirm that Progerin buildup causes damage and loss to enteric neurons and ICC leading to disruption in normal colonic motor activity and a huge distension in the colon wall. The development of a distended GI tract in Lmna^{HG-C} mutants reveals the importance of these cells in GI motor function and also offers a new model to study diseases related to Progerin formation in the GI tract.

Funding Sources: Supported by NIH DK57236 & PO1 DK41315.
**Title:** Weeks-Long Continuous Monitoring of Rodent Urodynamic Parameters Including Post Void Residual Volumes using a Novel Hybrid Physical-Computational Metabolic Cage System

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**Introduction/Objectives:** Rodent models are often used to study the long-term effects of invasive or exploratory therapeutic interventions on lower urinary tract (LUT) health. However, urodynamic parameters, such as voided volume and voiding efficiency (VE), can only be measured during brief windows each day because it is very time-intensive for researchers to collect this data. The goal for this work is to design and implement a system that allows 24-hour uninterrupted continuous monitoring of all essential urodynamic parameters in awake behaving rodents, while minimizing the need for human-conducted measurements and non-physiological experimental conditions.

**Methods:** We assembled a custom metabolic cage system that continuously houses rats for up to one month and monitors urodynamic parameters via custom integrated software. At the start of the study, rats are implanted with a flexible silicon suprapubic bladder catheter that is tunneled subcutaneously and externalized between the shoulder blades. Animals are then placed in the system which instantly separates solid and urine waste, records bladder pressure, and records voided volume. Twice daily, a researcher removed urine from the bladder for measurement, through the catheter without handling the animal. Using a computational optimization algorithm seeded with the measured data, we estimated with high accuracy the VE of every voiding event.

**Results:** We successfully recorded continuous urodynamics using the hybrid system for up to one month using 10 healthy control animals. The figure below shows our system capturing data from every single void made by one animal over the course of a week, voided volume (top) and VE (bottom). Our system accurately detects the behavioral differences during light and dark cycles, showing frequent low VE during dark phases (social urination) and infrequent high VE during the light phases when animals are dormant. Additional system validations in 4 animals show an average VE estimation error of 5%.

**Conclusions:** This system is capable of continuously recording urodynamic parameters for chronic studies without the need for continuous researcher presence. Moreover, the technology obviates the common, but non-physiological, steps of bladder infusion and frequent removal of residuals.

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Title: Behavioral monitoring and neuromodulation of feline bladder function

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Introduction/Objectives: Electrical stimulation of nerves, or neuromodulation, is a standard treatment for bladder problems after conservative approaches have failed. Many preclinical neuromodulation studies to study and optimize bladder control are performed using anesthetized animal models. We are investigating bladder activity with awake, behaving animals without the suppressing influence of anesthesia. Furthermore, we are examining the effect of nerve stimulation on behavior and bladder function in these awake animals.

Methods: During an aseptic surgery under isoflurane anesthesia, two single-lumen catheters were inserted into the bladder dome of adult, male, short-haired felines (N=4) via a laparotomy. In three cats a balloon catheter was also placed in the abdominal cavity. A bipolar stimulating cuff was placed around the left pudendal nerve via a postero-lateral incision. Catheters and stimulation leads were tunneled subcutaneously to a midline incision in the lower back. Catheter ports and electrode connections were housed within an enclosure mounted on the feline’s back. After a recovery period that included appropriate postoperative care, up to two test sessions were performed each week per cat across a period of 4-13 weeks. Test sessions in a ~3m open enclosure comprised of slowly filling the bladder (0.5-2mL/min) with body-temperature saline while concurrently monitoring pressures until the animal voided in a litter box. In a subset of trials electrical stimulation was applied to the pudendal nerve cuff (5-30 Hz; 0.25-2 x motor threshold). Post-session analyses include determining the interval between voiding events, peak voiding pressures, and voiding efficiencies.

Results: Across four cats, thirty-four sessions (6-140 minutes of continuous monitoring) were performed leading to 169 voiding events. Intervals between voiding events ranged from 2 to 31 minutes, dependent on varying factors including bladder inflammation (early post-surgery), infusion rate, animal size, and animal’s behavior. Animals tolerated the applied electrical stimulation amplitudes, preferring that the current is ramped up to a set level. In general, when stimulation was below the motor threshold lower voiding intervals were observed as compared to intervals with no stimulation. When the stimulation amplitude was above motor threshold the relationship varied. The stimulation frequency has not had a relationship to voiding interval. Other urodynamic parameters from these sessions are being reviewed.

Conclusions: This animal model allows for an opportunity to investigate bladder function and neuromodulation paradigms without the presence of anesthesia.

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S5A16

Title: Caspase signaling in ED patients and animal models

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Introduction/Objectives: Erectile dysfunction (ED) affects ~50% of men aged 40-70 and has a high impact on men’s health and quality of life. Current treatments are ineffective in the difficult to treat prostatectomy (16-82%) and diabetic (56-59%) patients due to injury to the cavernous nerve (CN), which provides innervation to the penis. With denervation the critical smooth muscle (SM) undergoes apoptosis and the penis becomes fibrotic, with increased collagen and a change in subtypes, thus altering the corpora cavernosal architecture. In order to devise novel and effective ED therapies, prevention of corpora cavernosal remodeling is critical. Apoptosis can take place via the intrinsic (caspase 9) or extrinsic (caspase 8) pathway. In this study we examine the caspase pathway by which apoptosis takes place in ED patients and in a CN injury rat model, in order to determine points of apoptosis intervention for therapy development.

Methods: Corpora cavernosal tissue was obtained from Peyronie’s, prostatectomy and diabetic ED patients (n=30) and immunohistochemical analysis was performed for caspase 3 cleaved, caspase 8 and caspase 9 (pro and active forms). Penis tissue from adult (P120) Sprague Dawley rats that underwent CN crush injury and were sacrificed after 1, 2, 4 and 9 days (n=16) also underwent caspase immunohistochemical analysis.

Results: Caspase 3 cleaved was observed at low abundance in corpora cavernosa from Peyronie’s patients, and at higher abundance in prostatectomy and diabetic tissues. Apoptosis takes place primarily through the extrinsic, caspase 8 dependent pathway in penis tissue of ED patients. In the CN crush rat model, caspase 3 cleaved was abundant from 1-9 days after injury. Apoptosis takes place primarily via the intrinsic caspase 9 dependent pathway after CN injury in the rat corpora cavernosa. Caspase 9 was first observed and most abundant in a proliferative layer under the tunica, and after several days, caspase 9 was observed in the lining of and between the sinuses of the corpora cavernosal tissue. Caspase 8 staining was observed initially at low abundance in the rat corpora cavernosa, and was not observed at later time points after CN injury (4 and 9 day).

Conclusions: Apoptosis takes place primarily through the extrinsic caspase 8 dependent pathway in ED patients, and via the intrinsic caspase 9 dependent pathway in the commonly used CN crush ED rat model. This is significant when considering points of intervention to suppress the apoptotic response to CN injury. Further study is required to determine if differences in rat and human ED apoptotic pathways derives from age of the tissues under study, since ED patients undergoing prosthesis implant to treat their ED, are typically older than commonly used rat ED models.

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S5A17

**Title:** Testosterone Replacement Enhances Internal Pudendal Artery Relaxation to Reverse Erectile Dysfunction in a Rat Model of Androgen Deprivation Therapy

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**Introduction/Objectives:** Many men undergo androgen deprivation therapy (ADT) to manage prostate cancer; however, erectile dysfunction (ED) and cardiovascular disease are common side effects. After men cease ADT treatment it may take years for testosterone (T) levels to recover. In some men, T levels fail to return to pre-ADT levels leaving them prone to cardiovascular disease and long-term ED. The internal pudendal arteries (IPA) supply blood to the penis and vascular injury to these vessels can lead to ED. This study will determine if castration impairs vascular function in systemic (aorta and mesenteric arteries) and penile vasculature (IPA), and if T can restore both erectile and vascular physiology. We hypothesize the IPA will develop severely impaired relaxation prior to systemic arteries following ADT, and T therapy will recover both vascular and erectile function.

**Methods:** Male Sprague Dawley rats (12 wks) were divided into 3 groups (n=8/grp): control (CON, no surgery), ADT (6 wks castration), and ADT+T (6 wks castration+T (3mg/kg) last 2 wks). Erections were assessed via cavernous nerve stimulation and measurement of intracavernosal to mean arterial pressures (ICP/MAP). Aortas, mesenteric arteries, and IPA were excised, cut into 2 mm segments and mounted into tissue baths. Contractility to high concentration potassium solution (KCl), electrical field stimulation (EFS), and phenylephrine (PE) were measured. Endothelial dependent acetylcholine (ACh) relaxation and endothelial independent relaxation to DEA NONOate were assessed. Non-adrenergic non-cholinergic (NANC) relaxation was evaluated in PE precontracted IPA rings by EFS in the presence of guanethidine and atropine. Total testosterone serum levels were measured with commercial ELISA.

**Results:** ADT impaired erectile function (ICP/MAP; CON: 0.83±0.03, ADT: 0.31±0.02; p<0.05) and IPA ACh relaxation (CON: 50.4±3.5, ADT: 31.2±2.3; p<0.05). In contrast, DEA NONOate relaxation was unchanged. ADT also impaired IPA NANC relaxation (CON: 40.8±5.1%, ADT: 22.1±3.9%; p<0.05). ADT did not impact IPA contraction to KCl, PE, and EFS. Interestingly, ADT did not change vascular contraction or relaxation in aortas or mesenteric arteries. Testosterone supplementation (T (ng/ml); CON: 4.2±0.8, ADT: 0.1±0.02, ADT+T: 32.9±3.2: p<0.05) restored erections (ICP/MAP; ADT+T 0.74±0.01 p<0.05) and improved ACh relaxation in IPA to values greater than CON (T: 63.9±2.9; p<0.05). Similarly, ADT+T enhanced IPA NANC relaxation to levels greater than CON (T: 69.2±1.4; p<0.05). Testosterone did not impact IPA contractions and had no effect on aortic or mesenteric vasoreactivity.

**Conclusions:** Our animal castration model demonstrates the development of ED within 6 weeks of castration. Additionally, there was evidence of pre-penile vascular dysfunction prior to decreases in systemic vascular function. T therapy recovers erectile function and greatly enhances IPA endothelial dependent and NANC-mediated relaxation. The safety of T therapy in cancer survivors remains a controversial topic, yet a growing body of literature is finding no correlation between T therapy and cancer progression. Consideration of T replacement therapy to restore erectile function in certain populations of prostate cancer survivors is warranted.

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SSA18

Title: Sonic hedgehog regulation of cavernous nerve regeneration and neurite formation in aged pelvic plexus

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Introduction/Objectives: Erectile dysfunction (ED) is a significant health concern that greatly impacts quality of life, and is common in men as they age, impacting 52% of men between the ages of 40 and 70. A significant underlying cause of ED development is injury to the cavernous nerve (CN), a peripheral nerve that innervates the penis. CN injury also occurs in up to 82% of prostatectomy patients. We recently showed that Sonic hedgehog (SHH) protein delivered by peptide amphiphile (PA) nanofiber hydrogel to the CN and penis of a prostatectomy model of CN injury, is neuroprotective, accelerates CN regeneration, improves erectile function ~60%, preserves penile smooth muscle 56% and suppresses collagen deposition 30%. This regenerative potential is substantial in an adult prostatectomy model (P120). However prostatectomy patients are typically older (61.5 ± 9.6 years) and our models should mimic patient conditions more effectively when considering translation. In this study we examine regenerative potential in an aged prostatectomy model (P200-329).

Methods: The caudal portion of the pelvic ganglia (MPG) and CN were dissected from adult (n=11), and aged (n=13) Sprague Dawley rats, and were grown in organ culture 3 days. Uninjured and 2 day CN crushed MPG/CN were exposed to Affi-Gel beads containing SHH protein, PBS (control), or 5e1 SHH inhibitor. Neurites were quantified by counting the number of growth cones normalized by tissue perimeter (mm) and immunohistochemistry for SHH, patched1 (PTCH1), smoothened (SMO), GLI1-3, and GAP43 were performed.

Results: SHH treatment increased neurites 3.5-fold, in uninjured adult, and 5.7-fold in aged rats. Two days after CN crush, SHH treatment increased neurites 1.8-fold in adult rats and 2.5-fold in aged rats. SHH inhibition inhibited neurite formation in uninjured MPG/CN but not in 2 day CN crushed MPG/CN. PTCH1 and SMO (SHH receptors), and SHH transcriptional activators/repressors, GLI1-3, were abundant in aged MPG/CN with unaltered localization. ROCK1 was induced with SHH treatment.

Conclusions: Reintroduction of SHH protein in an aged prostatectomy model is even more effective in promoting neurite formation/CN regeneration than in the adult. The first 48 hours after CN injury are a critical window when growth factors are released, that impact later neurite formation. These studies are significant because most prostatectomy patients are not young and healthy, as with adult rats, so the aged prostatectomy model will more accurately simulate ED patient response. Understanding how neurite formation changes with age is critical for clinical translation of SHH PA to prostatectomy patients.

Funding Source(s): NIH/NIDDK DK101536
Title: Targeted depletion of the microtubule regulatory protein fidgetin-like 2 (FL2) enhances axon regeneration and improves erectile function outcomes after cavernous nerve injury in a rodent model of radical prostatectomy.

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Introduction/Objectives: Erectile dysfunction is commonly experienced by patients who undergo radical prostatectomy and is thought to be caused by damage to the cavernous nerves (CN) during the surgery. We recently identified the microtubule (MT) regulatory protein fidgetin-like 2 (FL2) to be a promising therapeutic target for promoting cutaneous wound healing. Subsequently, we discovered that FL2 is also expressed in neurons, where it localizes to growth cones. Depletion of FL2 from cultured neurons with siRNA or by gene deletion significantly enhanced the rate of neurite growth. Given these observations, we hypothesized that targeted depletion of FL2 after CN injury would promote nerve regeneration and lead to improved erectile function following the injury. We tested this using a rodent model of radical prostatectomy.

Methods: Three rat models of CN injury were used: a mild injury, in which the nerves were crushed for 2 minutes with a smooth clamp; a moderate injury, in which nerves were crushed for 4 minutes with a serrated clamp; and a severe injury, where the nerves were transected. Immediately after injury, control or FL2 siRNA was directly applied to the wound. The siRNA was packaged into either a heparin sulfate microgel, which dissolves upon application to the wound, or encapsulated in nanoparticles which were pipetted onto the wound. Erectile function was assessed at several time points after injury by measuring the intracorporal pressure/blood pressure (ICP/BP) ratio following electro-stimulation of the CN.

Results: In all models of CN injury, FL2-siRNA treated animals had significantly higher ICP/BP ratios compared to controls beginning two to three weeks following injury. Remarkably, after CN-transection, there was visible regrowth of the CN at two weeks, with significantly improved erectile function compared to controls. Preliminary studies showing increased rates of neurite growth were repeated in vitro in adult sensory cultures, confirming a significantly enhanced rate of neurite outgrowth.

Conclusions: We have identified FL2 as a novel negative regulator of axon growth in neurons and a promising therapeutic target for promoting CN regeneration after injury. Our work indicates that depleting FL2 may promote nerve regeneration through directly enhancing the growth rate of injured axons, however, the impact of FL2 knockdown in non-neuronal cells at the injury site may play a critical role as well. Future work will further elucidate the mechanism(s) by which transient depletion of FL2 promotes nerve regeneration.

Funding Source(s): SMSNA “Scholar of Sexuality” graduate student fellowship; NIH RO1 GM109909; RO1 DK109314-01A1; NIH T32 5T32GM007491-39; New York State Spinal Cord Injury Research Board.
Title: Total Testosterone Level Correlates with Intraprostatic Lymphocyte Density

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Introduction/Objectives: Male hypogonadism promotes a pro-inflammatory state within the prostate which may drive prostatitis-like symptoms. Previous studies by our group demonstrated that hypogonadal men have increased histologic evidence of chronic prostate inflammation compared to eugonadal counterparts, however recent evidence suggests that higher levels of total testosterone (TT) may drive prostate inflammation as well. We examine the relationship between TT level and prostate lymphocytic infiltration.

Methods: Specimens from treatment naïve patients undergoing radical prostatectomy (RP) were retrospectively acquired. Patients matched for Gleason score, age, TNM stage were stratified by pre-RP TT: <293 ng/dL (hypogonadal), 293-464 ng/dL (low eugonadal), >464 ng/dL (high eugonadal). Quantitative analysis of lymphocyte density (cells/mm²) was performed with ImageJ. ANOVA and two-sided T-test were used to correlate lymphocyte densities with pre-RP TT, as well as other clinical factors associated with prostate inflammation.

Results: Twenty-three RP specimens (9 hypogonadal, 6 low eugonadal, 8 high eugonadal) were included. Median pre-RP TT was 170 ng/dL, 435 ng/dL, and 598 ng/dL in the hypogonadal, low eugonadal, and high eugonadal arms, respectively. When comparing tumor infiltrating lymphocyte (TIL) densities, there were significant differences between the three groups (393 cells/mm² in hypogonadal, 113 cells/mm² in high eugonadal, 27 cells/mm² in low eugonadal arms, respectively p=0.0356). Lymphocyte density in benign adjacent tissue was significantly higher in both hypogonadal and high eugonadal arms compared to the low eugonadal arm (310 cells/mm², 266 cells/mm², and 9 cells/mm², respectively, P = 0.445). There was no significant difference in infiltrating lymphocyte density between the hypogonadal and high eugonadal arms within benign tissue. There was a U-shaped relationship between infiltrating lymphocytes and TT level within both the tumor and benign tissue (Figure 1). Independent analysis using ANOVA revealed no association between infiltrating lymphocyte densities and co-existing benign prostatic hyperplasia, hypertension, hyperlipidemia and diabetes mellitus.

Conclusions: We demonstrate a U-shaped relationship between TT levels and intraprostatic lymphocyte infiltration within prostate tumor and benign adjacent tissue. These results are consistent with recent findings demonstrating reduced rates of prostatitis in patients with TT levels between 293-464 ng/dL. It is conceivable that this range may represent an optimal TT target for testosterone replacement therapy such that inflammation and prostatitis-like symptoms are reduced.

Funding: AUA Medical Student Research Fellowship, Alpha Omega Alpha Research Fellowship

Figure 1.
Title: Characterization of the Biaxial Biomechanical Properties of Human Post-Menopausal Prolapsed and Non-prolapsed Uterosacral Ligament

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Introduction/Objectives

More than 300,000 pelvic organ prolapse (POP) surgeries are performed annually in the United States alone, resulting in direct annual costs of over a billion dollars [1, 2, 3]. The uterosacral ligament (USL) is an important tissue which provides structural support to the female pelvic floor, without which POP would occur [4]. The aim of this study was therefore to determine and compare physiologically relevant biomechanical properties of healthy and POP-affected post-menopausal USL tissues.

Methods:

USL samples (Fig. 1A) were obtained from post-menopausal women with either a prolapse (n=13) or non-prolapse (n=7) condition (IRB Approved: 2017.016.A). Samples were stamped into a cruciform geometry and then speckle-coated with alcohol ink (Fig. 1B). After 10 equibiaxial preconditioned cycles, samples were loaded biaxially at 0.002/sec in the X- and Y-directions to different loading ratios (X:Y) of 1:0.5, 1:0.75, 1:1, 0.75:1 and 0.5:1. Fung-type constitutive model was fitted to the experimental curve [5].

Results:

The prolapsed USL exhibited a more nonlinear behavior and longer stretches (Figs. 1C and 1D). The non-prolapsed USL exhibited higher stresses, about 50% higher in both the X- and Y-directions, as well as stiffer behavior in both directions. Results indicate that prolapsed USL has anisotropic characteristics while non-prolapsed exhibited isotropic behavior.

Conclusions:

The Fung-type constitutive model was able to capture the experimental data. Perhaps, the nonlinear behavior exhibited in the prolapsed USL (particularly in Y-direction) is an indication of weakness. This is corroborated by its longer stretches as compared to the non-prolapse USL. Structure-function relationships will be determined in future.

Funding Sources: NIH grant P20GM103629 and Ochsner Translational Medicine Research Initiative.

Figure 1: USL specimen location and orientation (A), testing geometry with ink (B), biaxial mechanical results with Fung-type fits ((C) and (D))

Title: Hyperglycemia Induces Detrusor Hyperactivity by Altering the Caveolae-Dependent Regulation of ROCK Signaling Pathway

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Introduction/Objectives: Lower urinary tract symptoms are among the most prevalent complications of diabetes. In animal models of diabetes, the pathological phenotype of diabetic bladder dysfunction (DBD) includes a compensatory phase characterized by detrusor hypercontractility and is associated with an upregulation of Rho-ROCK signaling pathway. However, the mechanism by which the regulation of ROCK becomes altered with diabetes has not been fully elucidated. In other smooth muscle systems, ROCK signaling is regulated by caveolae, membrane microdomains that are involved in modulating contractile responses to a variety of stimuli. Although alterations in caveolae and caveolin proteins have been described with diabetes in several tissues including the bladder, the molecular relationship between caveolae and ROCK signaling under normal and diabetic conditions has not been investigated. The purpose of this study was to determine the functional effect of hyperglycemia on contractile responses in the bladder, and investigate whether caveolae mediated ROCK signaling underlies the bladder hyperreactivity induced by hyperglycemia.

Methods: Functional studies: Mouse bladder smooth muscle (BSM) tissues without mucosa were mounted in organ baths for isometric tension studies. The amplitude of bladder contractile responses to carbachol (CCh, 1μM), α-β-methylene-ATP (αβmeATP, 10μM) and KCl (120mM) were measured after exposure to 1, 2, 4 or 8 hours of high glucose (HG, 23mM). Appropriate time control experiments were generated either in euglycemic conditions (Kreb’s with 11.5mM glucose), or in Kreb’s supplemented with 11.5mM mannitol (to control for hyperosmotic conditions). In addition, the effect of exposure to HG for 2 hrs on the response to CCh was investigated in the presence of Rho kinase inhibitors (Y27632, 1μM and fasudil, 1μM) at sub-maximal doses that are without effect under euglycemic conditions, as well as after disruption of BSM caveolae by the cholesterol-depleting agent methyl-β-cyclodextrin (mβCD, 10mM, 1 hr). Molecular studies: Changes in protein expression of Rho, ROCK1 and ROCK2, as well as caveolin-1 (Cav-1) and caveolin-3 (Cav-3) were determined after 2 hrs of incubation in either HG, normal glucose, or mannitol in BSM by western blot (WB). The molecular interaction of Cav-1 and Cav-3 with ROCK1 and ROCK2 in BSM tissue was investigated by co-immunoprecipitation. The level of phosphorylation of myosin light chain (pMLC) after 2 hrs of HG in the presence of either Y27632 or mβCD was determined by WB.

Results: Starting at 2 hours of HG exposure, the amplitude of responses to CCh, but not to either αβmeATP or KCl, was significantly higher compared to those measured in respective time control experiments. Mannitol did not alter CCh responses at any time point, indicating that the increase in CCh contractions was not due to changes in osmotic pressure. The augmented contractions to CCh after 2hrs of HG was associated with increased pMLC levels in BSM. Moreover, the HG-induced increase in both CCh responses and pMLC levels were reduced by Rho kinase inhibitors. The expression of Cav-3, but not of Cav-1, increased after 2hrs of HG while neither Rho, ROCK1 nor ROCK2 expression was affected after 2hrs of HG. Both ROCK1 and ROCK2 coprecipitated with Cav-3 but not with Cav-1. The increase in CCh responses as well as the increase in pMLC induced by 2hrs exposure in HG was prevented after caveolar depletion.

Conclusion: Acute hyperglycemia increases the sensitivity to cholinergic stimulation in a ROCK and caveolae dependent manner. Rho-ROCK signaling in BSM is regulated by caveolae, as indicated by the interaction of Cav-3 with ROCK proteins. Thus, our findings suggest a novel molecular mechanism that links hyperglycemia-induced cholinergic hyperreactivity with facilitation of ROCK signaling by caveolae.
Title: Central (Spinal) Mechanisms of Persistent Bladder Pain: A role for MIF and HMGB1
Authors: Ma, F1,2, Meyer-Siegler, KL3, Leng, L4, Bucala, R4 and Vera, PL1,2
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Introduction/Objectives: Repeated intravesical PAR4 activation elicits persistent bladder hyperalgesia (BHA) lasting 5 days after the last treatment (Ma et al 2018). Persistent BHA was fully reversed by a systemic HMGB1 inhibitor while a MIF antagonist partly reversed it (Ma et al 2018). There is also growing evidence that spinal MIF and HMGB1 mediate inflammatory and neuropathic pain. We examined central (spinal) changes occurring during persistent BHA that may be responsible for maintaining bladder pain after resolution of bladder injury.

Methods: Persistent BHA (9 days) was elicited in female C57 mice by repeated (3x; 48 hr apart) intravesical instillation of PAR4-activating peptide (Ma et al 2018). On day 4, spinal (L6-S1; under isoflurane anesthesia) changed in MIF and HMGB1 were assessed with immunofluorescence, western blotting (WB) and RT-PCR, while glial activation was examined by immunofluorescence. Control mice received treatment with a scrambled peptide. On day 7, mice received an intrathecal (i.t.) injection of an HMGB1 inhibitor glycyrhrizin (25 µg in 5 µl of 5 % alcohol in PBS) and mechanical threshold. On day 9, mice were treated with vehicle i.t. injection as control.

Results: Immunofluorescence showed that MIF significantly increased while HMGB1 was decreased in the dorsal horn, dorsal grey commissure and intermediolateral area (areas receive bladder afferent information). In addition, c-fos (early gene) and Iba1 (microglial marker) were significantly increased in PAR4 treated mice. RT-PCR and WB showed no significant difference in spinal MIF and HMGB1 between PAR4 and scramble treated mice. I.t. treatment with HMGB1 antagonist significantly alleviated abdominal mechanical hypersensitivity at 1 and 2 hours and the analgesic effect was diminished at 6 hours (Fig 1). Vehicle treatment had no effect.

Conclusions: Persistent BHA is associated with spinal changes in MIF, HMGB1 levels and signs of glial activation. Furthermore, spinal treatment with HMGB1 temporarily reversed BHA. Our findings suggest that spinal MIF and HMGB1 participate persistent bladder pain induced by repeated intravesical PAR4.

Funding Source(s): NIH/DK0093496

Title: Age-Related Deficits in Sensory-Mediated Reflex Bladder Control in Rat

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Introduction/Objectives: The prevalence of lower urinary tract (LUT) dysfunction is highly correlated with age and the median age of the US population is projected to increase substantially; therefore, understanding the causes of age-related LUT symptoms is a major public health issue. The high rate of comorbidities in geriatric populations suggests that the emergence LUT dysfunction in this group is multifactorial in nature. However, this does not preclude the possibility that general age-related processes directly contribute to LUT symptoms. In this work, we assess the relationship between age and systems-level LUT function, and evaluate the hypothesis that age-related neuropathy plays a role in weakening reflexes that control healthy voiding behavior.

Methods: Using urethane-anesthetized female rats, we implanted a suprapubic bladder catheter used for intravesical saline infusion and a urethral catheter used to infuse saline into the proximal urethra and out the distal orifice. This preparation prevented bladder contractions from expelling urine through the urethra (which was blocked by the catheter), and allowed us to independently control the bladder infusion rate, bladder volume, and urethral flow rate. In three groups of animals, young (4-7 month), mature (12-15 month), and old (18-24 month), we systematically investigated LUT reflex responses to bladder filling and urethral infusion as a function of age. Bladder pressure, external urethral sphincter electromyogram, estrous, heart rate, and other physiological parameters were measured continuously during the acute experiments.

Results: With increasing age, the strength and sensitivity of LUT voiding reflexes were attenuated. Older animals were less likely to exhibit the augmenting reflex (AR, reflexive bladder contraction triggered by urethral stimuli to promote voiding) compared with younger animals exposed to the same magnitude stimulus (panel A). The switch from “continence-mode” to “voiding-mode” (i.e., activation of void inhibiting versus void promoting reflexes) was delayed in older animals, quantified using normalized bladder volume and reflex-evoked bladder contractions. Bladder pressures produced by AR-mediated contractions as well as bladder-distention evoked contractions were weaker in older animals (panel B).

Conclusions: The loss of reflex sensitivity in older animals indicates that urethral sensory neuropathy could be a contributing factor in age-related LUT symptoms. The failure of urethral infusion to evoke reliable and robust bladder contractions is suggestive of geriatric underactive bladder, which often presents clinically with the report of reduced urethral sensations in patients.

Funding Source(s): Wallace H. Coulter Foundation support.
**S6A25**

**Title:** Modulation of intracellular Ca\(^{2+}\) in interstitial cells of Cajal and smooth muscle cells by nitrergic neuromuscular transmission in the murine internal anal sphincter.

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**Introduction/Objectives:** Nitric oxide (NO) plays a fundamental role as an inhibitory neurotransmitter in the internal anal sphincter (IAS). Studies in other gastrointestinal (GI) muscles suggest that NO causes contractile inhibition by acting on ANO1\(^+\)/Kit\(^+\) intramuscular interstitial cells of Cajal (ICC-IM) or directly upon smooth muscle cells (SMCs). Nitrergic neuromuscular transmission (NMT) begins with an inhibitory junction potential (IJP) that occurs independently of voltage-dependent L-type Ca\(^{2+}\) channels (VDCCs). On the other hand, conduction of the IJP to adjacent cells closes VDCCs in SMCs causing contractile inhibition. In preliminary calcium imaging studies undertaken on the Kit-GCaMP6f mouse IAS we have found that there are two functionally distinct groups of ICC-IM. One group (Type II) generates large synchronized Ca\(^{2+}\) transients at the slow wave frequency and appear to be pacemaker ICC. The other group (Type I) displays activity similar to ICC-IM in other GI regions regions, i.e., asynchronous Ca\(^{2+}\) release events. The present study examined how nitrergic NMT in the IAS modifies Ca\(^{2+}\) transients in Type I\&II ICC-IM and SMCs and their dependence upon VDCC.

**Methods:** An inducible Cre/loxP technique was used to express a genetically-encoded Ca\(^{2+}\) biosensor (GCaMP) in a cell-specific manner. Calcium transients were imaged from the submucosal aspect of the IAS of mice expressing GCaMP6f in ICC (Kit-GCaMP6f; 20x) and GCaMP3 in SMCs (SM-GCaMP3; 4x) with a confocal spinning disc microscope (Yokogawa CSU X-1). Inhibitory motor neurons were stimulated with electrical field stimulation (EFS; 5 Hz, 10s) in the presence of atropine (1 \(\mu\)M), guanethidine (1 \(\mu\)M) and MRS2500 (1 \(\mu\)M).

**Results:** The present study shows that nitrergic NMT blocks Ca\(^{2+}\) transients in Type I ICC-IM in a VDCC-independent manner, i.e., NO-mediated inhibition of Ca\(^{2+}\) release from the endoplasmic reticulum. Since both IJPs and the actions of NO on Type I ICC-IM are independent of VDCC it provides further support for Type I ICC-IM as primary mediators of nitrergic NMT. Blocking Ca\(^{2+}\) release may lead to hyperpolarization in Type I ICC-IM by closing ANO1 channels. Conduction of this hyperpolarization to adjacent cells will then block Ca\(^{2+}\) transients in SMCs as well as in pacemaker Type II ICC-IM by blocking VDCC.

**Conclusions:** The present study shows that nitrergic NMT blocks Ca\(^{2+}\) transients in Type I ICC-IM in a VDCC-independent manner, i.e., NO-mediated inhibition of Ca\(^{2+}\) release from the endoplasmic reticulum. Since both IJPs and the actions of NO on Type I ICC-IM are independent of VDCC it provides further support for Type I ICC-IM as primary mediators of nitrergic NMT. Blocking Ca\(^{2+}\) release may lead to hyperpolarization in Type I ICC-IM by closing ANO1 channels. Conduction of this hyperpolarization to adjacent cells will then block Ca\(^{2+}\) transients in SMCs as well as in pacemaker Type II ICC-IM by blocking VDCC.

**Funding Source(s):** NIH DK078736
Title: Measurement of non-voiding rhythmic bladder contractions by M-mode ultrasound during urodynamics in humans

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Introduction/Objectives: Urodynamic tracings of patients with detrusor overactivity often demonstrate non-voiding rhythmic contractions. These rhythmic pressure changes are understood to be the result of the synchronization of contractions that also cause micromotion in the bladder wall. The goal of this study was to apply a non-invasive method to measure bladder wall micromotion previously optimized in a swine model to humans with and without urgency to determine its effectiveness in a clinically relevant setting.

Methods: A total of 28 women were prospectively recruited for extended ultrasound urodynamic studies. This included 14 women with no urgency symptoms and 14 women with significant urinary urgency as measured by the ICQ-OAB survey. After filling the bladder to 40% cystometric capacity, 85 second anatomical motion-mode (AMM) cine loops were obtained using a GE Voluson E8 ultrasound system with 8 MHz curved, abdominal probe. These images were imputed into a custom correlation-based texture tracking algorithm implemented in MATLAB to measure changes in the bladder wall thickness over time. The frequency characteristics of these thickness changes were compared to the frequency characteristics of the urodynamic vesical pressure tracings taken over the same 85 second period using Fourier transform analysis.

Results: Based on our previous porcine study and literature on rhythmic bladder contractions, significant bladder wall micromotion was defined as changes in wall thickness with peaks in the frequency range of 2-6 cycles/minute with amplitudes higher than 0.1 mm. Six of the 14 subjects with urgency and one of the 14 without urgency had micromotion meeting this criteria. There was a significant association of micromotion with urgency (Chi-square, p=0.029). All seven women with significant micromotion had a peak in the frequency domain of their pressure tracing within 20% of the micromotion frequency. This implies that the rhythm observed in the changes in bladder wall thickness correlate to the rhythmic changes in pressure within the frequency range of interest.

Conclusions: This study demonstrates the feasibility of a non-invasive method to measure bladder wall micromotion using transabdominal AMM ultrasound. Identification of a micromotion-associated subgroup of DO patients could enable better targeting of DO treatments for this group without the need for an invasive urodynamic study.

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Title: Selectively activating lower urinary nerves with epidural spinal cord stimulation

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Introduction/Objectives: One of the highest priorities for people with spinal cord injuries (SCI) is restoration of bladder control. Existing interventions to treat bladder dysfunction after SCI, including catheterization and pharmaceuticals, are inadequate, and it is therefore of interest to develop new therapeutic and technological approaches. Epidural spinal cord stimulation, a neuromodulation therapy in widespread use for intractable back pain, has recently been shown to provide significant motor control improvements in some people with spinal cord injury. In addition, these people have reported improvements in bladder and bowel function. We aim to investigate whether epidural spinal cord stimulation can directly recruit sensory neurons involved in lower urinary tract function, and if so, determine whether this stimulation can modulate functional micturition and continence behavior.

Methods: We placed custom multichannel epidural arrays (MicroLeads Inc and Ripple LLC) at several locations on the sacral spinal cord and cauda equina of anesthetized male cats. To measure antidromic compound action potentials evoked by stimulation, we placed nerve cuffs on the pelvic nerve and the pudendal nerve and its branches. Additionally, we placed a nerve cuff on the sciatic nerve to measure off-target effects of stimulation. Bladder and intraurethral pressures were monitored throughout the experiments. We stimulated at varying current amplitudes on the array to determine our ability to selectively recruit afferents from different nerves, the threshold at which each afferent was recruited and which nerves were commonly coactivated. Additionally, we stimulated in monopolar and multipolar configurations on the array to determine how current steering might be used to increase selectivity.

Results: We were able to selectively recruit each instrumented nerve while minimizing off-target effects. Using monopolar stimulation, nerve recruitment thresholds were lowest with the electrode placed over the S1 segment of the spinal cord (295±159 μA). At the S2 segment, mean threshold amplitude was 448±188 μA, and at S1, was 463±104 μA. Bipolar and tripolar electrode configurations required much higher stimulation amplitudes to recruit responses, with thresholds frequently greater than 1200 μA. The pelvic nerve was often coactivated with the main trunk and sensory branches of the pudendal nerve. While stimulation trains were optimized for measuring antidromic action potentials, we observed changes in intravesical and intraurethral pressure evoked by changes in stimulus location and amplitude.

Conclusions: We were able to selectively recruit nerves innervating the lower urinary using epidural spinal cord stimulation. Ultimately, we intend to use this selective stimulation to modulate function in the lower urinary tract. This could provide a new method to restore bladder control in people with SCI or other bladder dysfunction.

Funding: NIH SPARC award 1OT2OD024908 and Craig H. Neilsen Foundation, award 476681.
Title: Microstimulation of sacral dorsal root ganglia enables selective recruitment of sensory afferents of the lower urinary tract.

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Introduction/Objectives: Lower urinary tract (LUT) complications arising from idiopathic causes and secondary complications of injury and disease are of concern because of their immediate and lasting effect on a patient’s quality of life. Nociceptive and dysfunctional alterations to the sensory pathways innervating the urinary bladder and urethra have been implicated as drivers of overall bladder dysfunction. In order to investigate these sensory mechanisms, and examine the potential of neuromodulation of these sensory pathways as a therapeutic approach, we are interested in the extent to which selective recruitment of pelvic and pudendal nerve afferents is possible through microstimulation in the sacral dorsal root ganglia (DRG) using penetrating microelectrode arrays. We hypothesize that coordinated stimulation of these pathways could be used to elicit coordinated micturition reflexes to alter pathological functioning of LUT organs.

Methods: Male cats (n=4) were anesthetized with isoflurane (2%) and implanted with nerve cuffs (500-1000 μm, Micro-Leads Inc, USA) on the pelvic nerve and pudendal nerves branches. We also placed an intraurethral and a transvesical catheter to monitor bladder and urethral pressures. A laminectomy from L5-S3 was performed to expose S1, S2 and S3 sacral DRG. 32-channel Utah array (Blackrock Microsystems) were implanted into the DRG with a pneumatic inserter. Microstimulation was delivered through each electrode and antidromic compound action potentials were recorded at the nerve cuffs using a Grapevine neural signal processor (Ripple LLC). A binary search algorithm was used to determine the current threshold at which stimulation through each DRG microelectrode evoked a response in each nerve.

Results: Motor responses from the bladder, urethra and anorectal sphincter confirmed the accurate placement of implanted nerve cuffs. Microstimulation thresholds of each microelectrode were in the range of 2-50 μA. We found that the conduction velocities of the antidromic action potentials were consistent with the expected composition of these nerves and in the range of A\(^\delta\) (5-30m/s) and A\(^{\beta}\) (35-75m/s) group fibers. Pelvic afferents were predominantly A\(^\delta\), while pudendal afferents showed a mix of A\(^\delta\) and A\(^{\beta}\) fibers. Preferential recruitment of certain nerves were observed across the three DRG. The pelvic nerve was recruited in S1 and S2, while the caudal rectal and deep perineal nerves were observed in S2 and S3. Recruitment maps of all three DRG across suggest that all instrumented peripheral nerves can be selectively activated by the implanted microelectrodes, although the number of selective electrodes can vary significantly between spinal levels and animals.

Conclusions: We demonstrate selective recruitment of pelvic-parasympathetic and pudendal-somatic afferents innervating the LUT. Creation of recruitment maps revealed innervation patterns across DRG which may be able to activate reflex driven motor responses leading to micturition and continence reflexes.

Funding Source(s): This work was funded by NIH award NINDS R01 NS088184.
**Title:** Dynamic Time Warping Parameter Optimization for Bladder Event Detection Algorithm

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**Introduction/Objectives:** Real-time bladder event classification from single channel bladder pressure data is an integral component of a conditional stimulation system for treating urinary incontinence. Context Aware Thresholding (CAT) is a parameterized algorithm that enables accurate, real-time bladder event detection in the presence of motion artifacts. CAT’s detection accuracy can be improved by optimizing the parameter set for an individual using pre-recorded bladder pressure data. The ideal recording contains numerous fill/void cycles, but using this for optimization requires alignment of detected events with actual events in the presence of false positives. We propose an iterative training process using Dynamic Time Warping (DTW) for automatic event alignment, enabling more efficient training and parameter identification.

**Methods:** CAT algorithm tuning is an iterative process where parameters are modified, and the functionality is simulated in a real-time emulation environment. An optimal parameter set maximizes detection accuracy and minimizes the lag between event onset and detection. When using individual contraction recordings for training, samples are divided into training and validation sets. Typically, multiple iterations within the training set are needed to refine parameters and achieve >90% detection accuracy in the validation set. For recordings with numerous fill/void cycles, we propose the use of DTW to determine which simulated event corresponds to which annotated event in the presence of false positives. This enables the system to compute the average detection latency. After testing with one set of parameters, DTW is applied to the output, with the original data series serving as reference. If at least one warping path exists for which at least one event is successfully detected, the parameters and average lag time are stored. From this, the set resulting in the lowest average lag, fewest false negatives and false positives, and highest true positive rate, is selected.

The test dataset is comprised of 14 ambulatory recordings of bladder pressure from a male cat, with 32 voiding contractions recorded in total. For the experiment, 4 files were chosen at random and used for training, and the remaining 10 were used for validation. Contractions in the training set were divided into 10 separate contraction event files and used for the baseline optimization method. For DTW-based optimization, one of the four recordings was chosen, with the other 10 recordings used for validation. This was repeated with each training set recording.

**Results:** DTW-based optimization reduced optimization time by an average of 27% relative to the baseline iterative optimization with individual recordings, while maintaining the >90% detection accuracy threshold. The DTW-enabled optimization also resulted in 23% fewer false positives than the baseline optimization technique.

**Conclusions:** DTW-based optimization using extended recordings with multiple voids per recording is a promising technique for optimizing parameters for the CAT algorithm. It enables faster optimization times, while improving real-time classification performance.

**Funding Source(s):** The NIH SPARC program, NIH OT2OD023873.
Title: Aging related alterations of motor unit firing rate in the bulbospongiosus muscle using high density surface electromyography

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Introduction/Objectives: Previous studies have demonstrated that aging is associated with central nervous system deficits that disrupt descending excitatory drives to the limb muscles. However, no effort has been made to evidence whether similar effects exist in the pelvic floor muscles (PFM). This study aims to explore the effects of aging on the bulbospongiosus by assessing motor unit (MU) firing patterns using a non-invasive pelvic high-density surface EMG (HDsEMG) recording and analysis technique.

Methods: Healthy female young (n=6, 31.5±3.9 years) and elderly (n=7, 66.6 ±4.74 years) subjects, without a history of neurogenic disorders, were recruited to participate in this study. A 64-Channel (8x8) HDsEMG probe was inserted into the vaginal space for HDsEMG recordings. Resulting HDsEMG signals were decomposed into motor unit action potential (MUAP) spike trains using our previously developed k-means clustering convolution kernel decomposition (KmCKC) technique. The MUAP firing rate for each subject was defined as the average number of motor unit firing instances per second for all decomposed motor units. A linear regression model was employed to assess the relationship between the age and associating mean firing rate, and the p-value for the F-test of the model was recorded.

Results: EMG decomposition was successfully performed for all thirteen subjects. Mean MU firing rates were 12.73±2.1 and 10.45±1.5, for the young and elderly groups, respectively. A significant linear relationship (p<.045) was found between age and bulbospongiosus MU firing rate.

Conclusions: This study represents the first effort to examine the effect of aging on PFM MU firing rates using advanced non-invasive HDsEMG recording and analysis techniques. Our results demonstrated a strong negative correlation between age and PFM firing rate, signifying impaired descending excitation to the bulbospongiosus with advancing age. Our results also suggest the sensitivity and feasibility of using HDsEMG tools for further investigations of aging-related pelvic floor dysfunctions.

Funding Source(s): Funding support for this project was provided by NIH DK082644
Title: Comparison of systemic and penile vascular function in C57Bl/6N and C57Bl/6J mice following 12 weeks of high fat diet

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Introduction/Objectives: Vasculogenic erectile dysfunction has been reported in 79% of obese men. To model this pathology, C57Bl/6J (6J) mice are commonly fed a high fat diet (HFD) for 12 weeks. Unfortunately, this model fails to consistently produce an obese phenotype with vascular disease. Inconsistencies may be due to the current 6J strain developing over 200 genetic mutations from the original 6J mouse. A novel C57Bl/6N (6N) strain has been developed void of these mutations. The objective of this study is to evaluate the effects of HFD induced obesity on systemic and penile vascular reactivity in 6J and 6N mice.

Methods: Male 6J and 6N mice (10 wks old) were fed a 45% HFD or 10% fat control (CON) diet for 12 weeks (n=6 per group). Food intake and body weights were monitored weekly. Serial magnetic resonance images (MRIs) evaluated body composition and glucose tolerance tests were performed at 12 weeks. Aortas, internal pudendal arteries (IPA), and penises were excised and mounted in myographs (n=6 per group). Smooth muscle contractions were assessed via high potassium chloride (KCl), and concentration response curves (CRC) to phenylephrine (PE; α1 agonist) and endothelin 1 (ET-1). Endothelial independent relaxation was assessed using CRC to DEA NONOate (NO donor). Endothelial dependent relaxation to acetylcholine (Ach; muscarinic agonist) was also evaluated.

Results: Following 12 weeks of HFD, both mouse strains had increased body weight (p<0.05), increased body fat (P<0.05), and elevated fasting blood glucose (p<0.05) compared to age matched CON mice. KCl contraction was significantly decreased (p<0.05) in 12 week 6N HFD aortas, yet no KCl differences were observed in 6J aortas. Similar decreases in PE contraction (p<0.05) were noted in aortas of both strains following HFD. ET-1 contraction was not affected by HFD in 6N IPA, but increased in 6J IPA (p<0.05). Surprisingly after 12 weeks of HFD, endothelial dependent and independent relaxations were unchanged in both strains compared to age matched CON. No changes in penile vascular reactivity were evident in 6N or 6J mice.

Conclusions: Both male 6N and 6J mice fed a 12 week HFD develop marked increased adiposity and impaired glucose metabolism, yet neither strain exhibit penile vascular damage. Subtle changes in systemic and pre-penile contractility between the strains are evident with 12 weeks of HFD and may become more drastic with a longer HFD duration. These early changes warrant further evaluation of the phenotypic differences between 6N and 6J mice to identify underlying vascular mechanisms. Studying multiple strains may more closely approximate the genetic variations in humans and yield new therapeutic targets in the treatment of obesity induced vasculogenic erectile dysfunction.

Funding Source(s): SMSNA Research Grant to MRO, DiaComp Pilot and Feasibility Award to JLH.
PS32

Title: Quantitative morphometry assessment of elastic fibers in pelvic organ prolapse

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Introduction/Objectives: Aberrations of the elastic fibers, extracellular matrix (ECM) structures that provide elastic stretch and recoil properties to tissues is a potential pathophysiology behind pelvic organ prolapse (POP) development, which compromises human health and quality of life. There is a significant void in methods to characterize and hence assess the quality and quantity of new elastic fiber assembly and to investigate structural changes in the elastic matrix. Therefore, in this study we have developed and validated a technique involving Image Pro® based morphometric analysis of elastic fibers on modified Hart stained tissue sections and used this method to assess the effect of elastogenic factors (EFs) treatment on vaginal tissue cultures from LOXL1 KO (Lysyl oxidase like -1 Knock Out) prolapsed mice.

Methods: Whole vaginal tissues from Wild type (WT), LOXL1 KO multiparous prolapsed (MP) and LOXL1 KO multiparous non-prolapsed (MNP) mice were harvested via a midline abdominal incision. ¼ of each vagina was fixed immediately after harvesting in 4% Paraformaldehyde (PFA) (D0 tissues) and another ¼ was flash frozen. For WT and MNP the remaining vagina was cultured as a single piece for 3 weeks in DMEM/F-12 containing 10% v/v Fetal Bovine Serum and 1% v/v Pen Strep. For MP, the vagina was bisected, and ⅔ was cultured as above (Untreated case). The other ⅔ was cultured for 3 weeks in medium supplemented with EFs (5 ng/ml of Transforming Growth Factor-β1 and 2 ug/ml of Hyaluronan Oligomer) (Treated case) and fixed in 4% PFA. The fixed tissues were paraffin embedded, sectioned and stained with modified Hart’s stain (1 volume of Wieger’s iron resorcin fuschin and 9 volumes of 1% hydrochloric acid in 70% ethanol) which stains elastic fibers in purple. The stained sections were scanned using a whole slide scanner and morphometric analysis was performed on each section using Image Pro® Plus software. Data was analyzed using a random effect statement with a nesting of repeated measurements within each sample (3 per animal) where the samples were nested within each animal and p < 0.05 was taken to indicate a statistical significance between groups.

Results: No significant differences were observed in the measured parameters between MNP and MP in D0 tissues, however significantly higher mean aspect ratio, maximum diameter and perimeter length was observed in MP compared to MNP after 3 weeks of culture. Treatment of MP tissues in culture with EFs caused a significant increase in the mean area and perimeter length of elastin fibers. Similarly, there was also significant increase in total percent area of elastin and variability in area, maximum diameter and minimum diameter in MNP. No significant differences were observed in measured parameters between WT and MNP in D0 tissues, but a significantly higher mean aspect ratio was observed in MNP compared to WT in 3 weeks culture.

Conclusions: This technique is useful in describing the characteristics of elastic fibers and the changes that occurs in them with POP. This technique can be very useful in tissue engineering to specify structural changes of the tissue with treatment.

Funding Source(s): NIH R21 HD078820
Title: Effects of elastase digestion on the murine vaginal wall biaxial mechanical response

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Introduction/Objectives: Although the underlying mechanisms of pelvic organ prolapse (POP) remain unknown, disruption of elastic fiber metabolism within the vaginal wall extracellular matrix has been highly implicated. It has been hypothesized that elastic fiber fragmentation correlates to decreased structural integrity and increased risk of prolapse; however, the mechanisms by which elastic fiber damage may contribute to prolapse are poorly understood. Further, the role of elastic fibers in normal vaginal wall mechanics has not been fully ascertained. Therefore, the objective of this study was to investigate the contribution of elastic fibers to murine vaginal wall mechanics.

Methods: Vaginal tissue (n=8) from C57BL/6 female mice were mechanically tested following Tulane Institutional Animal Care and Use Committee approved biaxial extension-inflation protocols before and after intraluminal exposure to elastase. Changes in area fractions of elastin, collagen and smooth muscle cells were assessed from histological images. To assess changes in mechanical properties, a microstructurally motivated strain-energy function was implemented in a hyperelastic, transversely isotropic framework. Pre- and post-digestion optimized model parameters, average compliance and histological area fractions were compared using Student’s t-tests (significance: p < 0.05, trend: 0.05 < p ≤ 0.1).

Results: Elastase digestion induced changes in the vaginal geometry, as evidenced by increased outer diameter (p < 0.06), and reduced wall thickness (p < 0.09). Additionally, elastase treatment induced stiffer biaxial mechanical response (reduced distensibility) of the tissue, particularly at higher loads. Additionally, the constitutive modeling results suggest that collagen fibers within the vaginal wall are primarily oriented diagonally with a slight preference towards the circumferential direction. A reduction in average compliance of the vaginal vessel was observed at 5 and 10 mmHg (p < 0.05). From histological area fraction measurements, a statistically significant (p < 0.03) lower elastic fiber area fraction was observed after treatment (before: 2.4%, after: 0.9%).

Conclusions: Overall, these results suggest that elastic fibers may play an important role in vaginal wall mechanical function—providing compliance and stabilizing collagen fiber crimp, thereby regulating collagen fiber recruitment with increasing load. These findings may be important towards understanding the underlying structural and mechanical mechanisms of POP, and aid in the development of growth and remodeling models for improved assessment and prediction of changes in structure-function relationships with prolapse development.

Funding Source(s): Newcomb College Institute (NCI) Faculty Grant, National Institutes of Health (NIH) P20GM103629, and National Science Foundation (NSF) Early Faculty CAREER Development Award (CMMI-1751050)
Title: Tonic inhibition of murine proximal colon contractions is due to nitrenergic suppression of Ca^{2+} release events coupled to activation of Ano1 in interstitial cells of Cajal

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Introduction/Objectives: Contractions of the proximal colon are controlled by intrinsic and extrinsic motor neurons that influence the functions of an electrical syncytium of cells; smooth muscle cells (SMCs) that generate the forces of colonic motility, interstitial cells of Cajal (ICC) that generate pacemaker activity and transduce neural inputs, and PDGFRα² cells that also transduce neural inputs. ICC regulate colonic motility through Ca^{2+} entry and release events that activate Ca^{2+}-activated Cl⁻ channels (encoded by Ano1). Ano1 mediated inward currents, or suppression of these events, tune the excitability of colonic SMCs. Under resting conditions, tonic neural inhibition suppresses the tendency for SMC contraction in the proximal colon in many species. To date, the post-junctional effector cell(s) and intracellular pathways responsible for mediating tonic inhibition are unknown. We sought to investigate if tonic inhibition of the proximal colon is mediated by influencing Ano1 activation in ICC.

Methods: Muscles of the proximal colon were dissected from 10-14 week old C57 mice. Strips of proximal colon oriented in the circular axis were mounted in contractile organ baths and fastened to a force transducer and contractions were recorded using AcqKnowledge software.

Results: Proximal colon contractions are limited by tonic neural inhibition, as blocking enteric neural input with tetrodotoxin (TTX, 1μM) unleashed significant increases in colonic contractions. This effect was mimicked by an inhibitor of nitric oxide (NO) synthesis, Nω-Nitro-L-arginine (L-NNA; 100μM) or by an inhibitor of soluble guanylate cyclase, ODQ (10μM). In contrast, blocking purinergic neurotransmission with MRS 2500 (1μM) had little effect on muscle contractions. The excitatory effects of TTX, L-NNA and ODQ were inhibited by the Ano1 antagonists, benz bromarone (0.3-3μM), Ani9 (0.3-3μM) and CaCC-inh A01 (1-10μM). These observations are consistent with the idea that NO mediates tonic inhibition in the proximal colon by ongoing suppression of Ano1 currents. This conductance is activated in the absence of nitrenergic input, causing enhancement in smooth muscle excitability and contraction. This was supported by the observation that a hyperpolarizing influence produced by pinacidil (0.3-3μM; an activator K_{ATP} channels) reversed the excitatory effects of TTX, L-NNA and ODQ. Ano1 is expressed exclusively in ICC in GI muscles, and its activation is due to Ca^{2+} release events in these cells. Blocking Ca^{2+} release in ICC by blocking intracellular stores with the SERCA pump inhibitor thapsigargin (1-10μM) or preventing store refilling with the store-operated Ca^{2+} entry channel blocker GSK 7975A (1-10μM) reversed the excitatory effects of TTX, L-NNA and ODQ.

Conclusions: Tonic inhibition of murine proximal colon contractions is due to nitrenergic suppression of Ca^{2+} release events that are coupled to activation of Ano1 in ICC.

Funding Source(s): NIH / NIDDK P01 DK 41315 and a Physiological Society Undergraduate Summer Research Grant.
Title: The β3-adrenoceptor agonist isoproterenol attenuates detrusor smooth muscle cell excitability and spontaneous contractility by activating Kv7 channels in guinea pig urinary bladder.

Authors: Sarah Maxwell, John Malysz, Viktor Yarotskyy, and Georgi V. Petkov

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Introduction/Objectives: β-Adrenoceptors regulate urinary bladder function. Their pharmacological activation can promote urinary bladder storage via relaxation of detrusor smooth muscle (DSM). The underlying mechanisms involve both ion channel-dependent and -independent targets. New evidence suggests that in vasculature, activation of β-adrenoceptors causes potentiation of Kv7 channel activity and decrease in contractility via adenylate cyclase (AC)-cAMP-Protein Kinase A (PKA) pathway. Our group has recently revealed a key physiological role for the Kv7 channels in DSM function. The aim of this study was to determine whether the effects of β3-adrenoceptors on DSM excitability and contractility involve Kv7 channels.

Methods: Urothelium-free DSM strips were obtained from adult male guinea pigs and used in isometric tension recordings and for enzymatic isolation of single DSM cells utilized in amphotericin perforated patch-clamp recordings. The effects of the Kv7 channel inhibitor XE991 (10 μM) were examined on: (1) isoproterenol (ISO, 3 μM)-evoked hyperpolarization in freshly-isolated single DSM cells, (2) ISO-mediated relaxation of spontaneous phasic, KCl (20 mM)-induced, or muscarinic agonist carbachol (CCh, 1 μM)-induced contractions in DSM strips, and (3) forskolin-induced inhibition of spontaneous DSM phasic contractions.

Results: In single DSM cells, ISO induced 8.3±2.9 mV hyperpolarization (n=7), which was reversible upon washout (6.7±4.5 mV depolarization, n=3). In the presence of ISO, XE991 caused 6.6±2.3 mV depolarization (n=3). In DSM cells pre-treated with XE991, ISO did not change the membrane potential (n=5). In DSM tissue strips, ISO concentration-dependently attenuated spontaneous, KCl-induced, and CCh-induced phasic contractions with similar potencies for amplitude, force, duration, and frequency (IC50’s: ~100-1000 nM), and maximum inhibition efficacies of ~60-90% for spontaneous (n=10) and ~20-50% for KCl- or CCh-induced phasic contractions (n=10-12). In the presence of XE991, maximum efficacies for ISO-mediated inhibition of spontaneous contractions lowered to ~50% (control: ~85%) for amplitude and force, and to ~20% (control: ~60%) for duration (n=9). XE991 did not alter the potencies and maximum efficacies for KCl- and CCh-induced phasic contractions (n=9-11). Forskolin concentration-dependently caused inhibition of spontaneous phasic contraction parameters with similar potencies and efficacies (IC50’s: ~1-3 μM, maximum inhibitions: ~40-80%; n=23). XE991 did not change the effects of forskolin on DSM phasic contraction amplitude, force, or duration (n=22).

Conclusions: The pharmacological activation of β-adrenoceptors with ISO induces DSM cell hyperpolarization and inhibition of spontaneous phasic contractions in tissue strips via activation of Kv7 channels. The mechanism for β-adrenoceptor regulation of Kv7 channels in guinea pig DSM does not involve the AC-cAMP-PKA pathway.

Funding Source: NIH R01 DK106964 to Georgi V. Petkov.
PS36

Title: Impact of nanotechnology in the post-surgical treatment of infection and inflammation in penile implants.

Authors: Moses Tar1, Guillermo Villegas1, Augene Park1, Andrew Draganski2, Girish V. Mavelli3, Joel Friedman3, Joshua Nosanchuk4 Pedro Maria1 and Kelvin Davies1,3,

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Introduction/Objectives: Around 25,000 surgical penile implant procedures are performed in the US every year of which about 5% are associated with complications (mainly infection and inflammation). The aim of the present study was to determine if application of a novel nanoparticle delivery system for nitric oxide (NO-np) or curcumin (curc-np) reduced infection rates and modulated inflammatory markers in a rat model of infection resulting from implants.

Methods: Penile implant components were supplied by Coloplast Inc. as 36 (2 mm by 6 mm) segments of silicone (tubing) and 36 (2 mm by 6 mm) segments of Bioflex (cylinder). Rats were divided into the following groups (1) Control animal; sterile implants; no np; (2) Sterile implant; treated topically with blank-np, (3) Sterile implant treated topically with curc-np, (4) Sterile implant treated topically with NO-np, (5) Control animal; S. aureus contaminated implants; no np, (6) S. aureus contaminated implants; treated topically with blank-np, (7) S. aureus contaminated implants; treated topically with curc-np, (8) S. aureus contaminated implants treated topically with NO-np. Animals were anesthetized and implants inserted sub-dermally and secured with a single stitch. Immediately after surgery, and everyday 2-days up to 10 days np (200 mg) were applied to cover the site of implant as a coconut oil paste. At 10 days animals were euthanized and infection determined by analyzing plaque forming units and expression of inflammatory markers assayed by quantitative RT-PCR.

Results: Visual assessment revealed reduced redness and swelling of the implant areas in controls compared to animals receiving the different np. Interestingly, we observed improved hair regrowth in the animals treated with curc-np. With non-contaminated silicone implants, (groups 1-4) there was an unexpected increase in infection rates in all groups treated with np (41-fold, p<0.01) which was not a result of contaminated np which we confirmed were sterile. However, there was a significant decrease in the infection rates associated with the silicone implants with the NO-np and curc-np treated animals compared to blank-np. The bioflex implants showed a similar trend, but variability prevented this reaching significance. Both Curc- and NO-np caused treatment-specific modulation of inflammatory marker expression.

Conclusions: In these experiments, treatment with blank-np, NO-np and curc-np did not improve infection rates in animals receiving sterile implants. However, there was a significant decrease in the infection rates associated with implants with the NO-np and curc-np treated animals compared to those treated with blank-np. Blank-np seemed to mute the inflammatory response, whereas curc-np and NO-np modulated mRNA expression of inflammatory markers. Additional work would be required to conclusively determine the efficacy of NO- and curc-np in reducing infection or inflammation associated with penile implants.

Funding Source(s): Coloplast Inc., Zylo Therapeutics Inc.
Title: Biaxial Contractile Response of Murine Vaginal Tissue

Authors: Gabrielle L. Clark1*, Dylan J. Lawrence1, Sarah H. Lindsey2, Laurephile Desrosiers3, Leise R. Knoepf3, Carolyn L. Bayer1, Kristin S. Miller1

Affiliations: Department of Biomedical Engineering, Tulane University, New Orleans, USA1, Department of Pharmacology, Tulane University, New Orleans, USA2, Department of Female Pelvic Medicine and Reconstructive Surgery, Ochsner Clinical School, New Orleans, USA3

Introduction/Objectives: One in ten women will require surgical intervention for pelvic organ prolapse (POP), characterized as the descent of the pelvic organs and their protrusion through the vagina. Studies show that women with POP display a decrease in vaginal smooth muscle cell composition and contractile function. Prior investigations of vaginal contractility have been performed independently in the circumferential or longitudinal direction, however the vagina is under multiaxial loading which may affect the contractile response. The objective of this study was to assess the contractile response of the murine vagina under various physiologically-relevant biaxial loading conditions. We hypothesized that the contractile response decreases with increased loading.

Methods: Vaginal tissue from female C57BL/6 mice 4-6 months of age at estrus (IACUC approved) was freshly isolated and the intact tissue was secured in an inflation-extension device. At a fixed physiologically-relevant length (n=5), the tissue was subjected to various pressures and contracted with 40mM of KCl. Further, at the measured mean in vivo pressure (7 mmHg; n=5) the vagina was subjected to various lengths, followed by contraction. The outer diameter was optically tracked and axial force measured with a transducer. A one-way ANOVA with Bonferroni correction was performed to identify differences in contraction based upon load (p<0.05).

Results: The magnitude of circumferential contraction was diminished at 15 mmHg (Fig 1A). No significant change in axial force was detected with loading (Fig 1C&D).

Fig 1. Biaxial contractile response of murine vaginal tissue. Change in outer diameter with contraction at various (A) physiologically-relevant pressures and (B) lengths. Change in axial force with contraction at various (C) physiologically-relevant pressures and (D) lengths. Magnitude of circumferential contraction was diminished at higher pressures. Data is reported as mean ± standard error of mean. * p<0.05.

Conclusions: This study simultaneously assessed circumferential and axial contraction of the vagina under various physiologically-relevant biaxial loading conditions. These results demonstrate that there was a decrease in circumferential contraction as a function of increasing pressure. This may indicate that increased intraabdominal loads, which is a risk factor for POP, may affect the vagina contractile response. Methods used herein may provide a better insight into vaginal contractility and further investigation of pharmacological interventions.

Funding Source(s): NSF CAREER Award Program- CMMI-1751050 (Tulane University)
Title: Nicotinic receptors on nerve terminals induce acetylcholine but not ATP release in canine bladder

Authors: Alan S. Braverman¹, Nagat Frara¹, Danielle M. Salvadeo¹, Mary F. Barbe¹ and Michael R. Ruggieri, Sr.¹,²

Affiliations: ¹Department of Anatomy and Cell Biology, Lewis Katz School of Medicine at Temple University USA, ²Shriners Hospitals for Children of Philadelphia, Philadelphia, PA.

Introduction/Objectives: Carbachol, a mixed muscarinic and nicotinic agonist similar to acetylcholine, is often used for in-vitro bladder contraction with the implicit assumption that it causes contraction by only activating bladder smooth muscle muscarinic receptors. We sought to determine whether nicotinic receptors may also be involved in canine detrusor muscle contractions in vitro.

Methods: Mucosa denuded female canine bladder muscle strips from sham operated animals were used from a larger study of nerve transfer for bladder reinnervation. Strips were fixed between force transducers and positioners and suspended in Tyrode’s solution bubbled with 95% O₂/5% CO₂ at 37°C. After stretching to a length of optimal force production, maximal responses to 120 mM KCl were determined then antagonists were added for 20 minutes before inducing contraction with the nicotinic agonists epibatidine and nicotine itself.

Results: Epibatidine induced contractions that were 40% of the maximal response to 120 mM KCl whereas nicotine only induced contractions that were 20% of KCl. The muscarinic receptor antagonist atropine (10 μM) completely blocked 10 μM epibatidine or 1 mM nicotine induced contractions but desensitization of purinergic receptors with 10 μM α,β methylene ATP did not. Blocking sodium channels with 1 μM tetrodotoxin (TTX) had no statistically significant inhibitory effect on epibatidine or nicotine induced contractions. Desensitizing nicotinic receptors by exposure to nicotine blocked contractile responses to epibatidine and vice versa. The skeletal muscle neuromuscular junction nicotinic receptor antagonist atracurium besylate (5 μM) blocked both epibatidine and nicotine induced contractions. Epibatidine contractions were also completely blocked by another skeletal muscle neuromuscular junction nicotinic receptor antagonist tubocurarine (1 μM) or the ganglionic nicotinic antagonists hexamethonium (100 μM) or mecamylamine (10μM).

Conclusions: Because of atropine blockade but not blockade by α,β methylene ATP desensitization, the nicotinic agonists induce bladder contractions indirectly by releasing acetylcholine but not ATP from intramural nerve terminals. Because TTX was ineffective, these nicotinic receptors do not need to induce action potentials in the nerve terminals and thus are likely located near the neuromuscular junction of the nerve terminals. The nature of these nicotinic receptors appears to be somewhat unusual in that they can be blocked by antagonists thought to be selective for skeletal muscle neuromuscular junction nicotinic receptors (atracurium and tubocurarine) as well as ganglionic nicotinic receptors (hexamethonium and mecamylamine).

Funding Source(s): NINDS R01NS070267 to MRR and MFB.
**Title:** Eugonadal Testosterone Levels Positively Regulates Erectile Function in Isolated Human Corpus Cavernosum

**Authors:** Laith Alzweri¹, Serap Gur², Ecem Kaya-Sezginer², Didem Yilmaz-Oral², Asim Abdel-Mageed¹, Wayne J.G. Hellstrom¹

**Affiliations:** ¹Departments of Urology and Pharmacology, Tulane University Health Sciences Center, New Orleans, LA, USA. ²Department of Pharmacology, School of Pharmacy, Ankara University, Turkey

**Introduction/Objectives:** Testosterone (T) deficiency (hypogonadism) is associated with erectile dysfunction (ED). The relaxant response of T on non-genomic pathways on the corporal smooth muscle has been reported, but the in vitro effects of T on human corpus cavernosum (HCC) have not been documented. We aimed to compare the mediating effects of different concentrations of T on nitric oxide (NO)-dependent and independent nitrergic relaxations in organ bath studies and the mode of action targeting the cavernous NO/cyclic guanosine monophosphate (cGMP) pathway.

**Methods:** HCC samples were obtained after consent from men undergoing penile prosthesis implantation (n = 9). After phenylephrine (Phe) contraction, electrical field stimulation (EFS), acetylcholine (ACH) and PDE-5 inhibitor (sildenafil) induced relaxation at 150, 400 and 600 ng/dL T incubations of HCC strips were performed using organ bath preparations. HCC measurements of endothelial NO synthase (eNOS), neuronal (nNOS) and PDE5 were evaluated through immunostaining, Western blotting, and cGMP and nitrite/nitrate assays.

**Results:** The relaxation responses to ACh and EFS in isolated HCC were significantly increased at all T levels as compared to untreated tissues. However, sildenafil induced relaxant responses were significantly increased at eugonadal (E) T. Unaltered neurogenic contractions to EFS were observed. E T levels may be accompanied by increased eNOS, nNOS and cGMP, along with lower PDE5 protein expression. Tissue nitrate/nitrite (NOx) concentration (NO production marker) were enhanced by E T levels. (Table 1)

**Conclusions:** We provide novel data that reveals the role and importance of the short-term and modulatory effects of T incubation in HCC. E T levels indirectly and specifically mediated HCC relaxation via downstream stimulation of nNOS, eNOS and cGMP and by inhibiting PDE5, causing restoring of erectile function. T replacement therapy may upregulate erectile function by modulating endothelial function in hypogonadal men with ED, and improve the therapeutic response of PDE5i. Additional studies are required to establish the nongenomic effects of T to maintain erectile function.

**Funding Source(s):** None

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**Table 1**

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<th>Untreated</th>
<th>Hypogonadism (150 ng/dL)</th>
<th>Eugonadism (400 ng/dL)</th>
<th>Hypergonadism (over 600 ng/dL)</th>
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<td>% Relaxation of Phe contraction (Emax)</td>
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<td>ACh (100 μM)</td>
<td>46.3 ± 5.6</td>
<td>74.5 ± 6.1*</td>
<td>93.7 ± 3.3***</td>
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<td>EFS (20 Hz)</td>
<td>26.0 ± 5.1</td>
<td>44.0 ± 4.0*</td>
<td>56.6 ± 9.2*</td>
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<td>Sildenafil (10 μM)</td>
<td>14.2 ± 3.7</td>
<td>26.5 ± 12.5</td>
<td>95.0 ± 2.9***</td>
<td>82.8 ± 9.6***</td>
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<td>EFS (40Hz)</td>
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<td>cGMP (pmol/g²)</td>
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<td>26.10 ± 4.3**</td>
<td>31.60 ± 4.33**</td>
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<td>NO₂/NO₃ (NOx, nmol/g tissue)</td>
<td>70.13 ± 3.26</td>
<td>62.6 ± 2.18</td>
<td>98.10 ± 5.60*</td>
<td>87.05 ± 26.25*</td>
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</table>
Title: Collagenase clostridium histolyticum (CCH) attenuates human corpus cavernosum contraction in vitro.

Authors: Laith Alzweri¹, Serap Gur², Sudha Talwar¹, Suresh Sikka¹, Asim Abdel-Mageed¹, Wayne Hellstrom¹

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Introduction/Objectives: Collagenase clostridium histolyticum (CCH), marketed as Xiaflex® (Endo Pharmaceuticals, Inc., Malvern, PA, USA) is an effective and safe minimally invasive intraleisional treatment option for Peyronie’s disease (PD). A published retrospective clinical study showed non-significant effect of CCH on penile vascular parameters, or the International Index of Erectile Function (IIEF) score in Peyronie’s patients who completed four rounds of CCH when compared to baseline. Although PD is strongly associated with erectile dysfunction, there are no studies related to the understanding of such effects of CCH on human corpus cavernosum (HCC) smooth muscle function. Our aim was to explore the effects of different concentrations of CCH on nitric oxide (NO)-dependent and -independent relaxations of HCC in organ bath studies to understand if CCH can improve HCC relaxation and thus ED in PD patients.

Methods: HCC samples were obtained from men undergoing penile prosthesis implantation (n = 10). After phenylephrine (Phe) contraction, electrical field stimulation (EFS), and acetylcholine (ACh) induced relaxation at [0.23 and 0.9 mg] CCH incubations of HCC strips were performed using organ bath preparations. HCC measurements of endothelial NO synthase (eNOS), neuronal (nNOS) and VEGF were evaluated through immunostaining and Western blotting.

Results: Various doses of CCH did not reduce the maximal contractile response of Phe and the relaxant response to electrical field stimulation (EFS, 20Hz) in HCC. Pre-incubation with CCH significantly reduced contractile tension evoked by EFS (80 Hz) by 37.5%, and increased Acetylcholine (ACh)-induced relaxation (10-3M) by five-fold at [0.23 and 0.9 mg].

Conclusions: CCH may have a potential relaxant effect on HCC tissues, which may be attributed to the blocking of sympathetic adrenergic receptors resulting in reduced EFS-induced contraction (80Hz) and enhancing parasympathetic cholinergic response. The relaxation response to CCH is likely to be dependent of NO-cGMP pathway. Although incubating HCC with CCH (0.9 mg) may have in vitro pro-erectile effects on the penile vasculature, this was not shown in a clinical setting using CCH at 0.58 mg dose. This adds to the safety profile of CCH, and more studies are required to examine the therapeutic potential of CCH pro-erectile effects with increased dosing in vivo to better address PD and the associated ED.

Funding Source(s): None
Title: Ovine Model for Preclinical Urological Research: Extending the Large Animal Model to Bladder Hyperactivity?

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Introduction/Objectives: A preclinical model exhibiting symptoms consistent with the clinical manifestation of overactive bladder (OAB) would allow evaluation of the impact of sacral neuromodulation (SNM) on bladder dysfunction, thus improving our ability to identify and translate clinically meaningful therapeutic concepts for treatment of OAB. Recently published work reports use of the vitamin A derivative retinyl acetate (RA) to cause bladder hyperactivity in rodents (Wróbel, A, Lancut, M, Rechberger, T; J Pharmacological Toxicological Methods 74 (2015) 7–16). Importantly, introducing this compound into the bladder did not cause non-specific irritation and damage to the urothelium, consistent with the clinical diagnosis of non-neurogenic OAB and in contrast to acetic acid-induced bladder hyperactivity models. This study aimed to implement a RA-induced bladder hyperactivity model in the established sheep model (Brink, T, Zimmerman, PL, Mattson, M, Su, X., Nelson, D; J Urol. 2015 Jul;194(1):252-8) to enable subsequent study of neurostimulation treatment effects on bladder dysfunction through the application of cystometry and neuromodulation.

Methods: During initial screening studies, instillation of RA into the bladder appeared to cause a decrease in bladder capacity (BC). To obtain a systematic characterization of the effect, this study performed weekly intravesical instillations of RA and control solutions in four, female, Polypay sheep. A stable formulation of 1% w/v RA (or vehicle solution) was prepared within 24 hours of use for each experiment. Instillation of solutions into the bladder was performed by pump-controlled infusion through a urethral catheter (three sequential fills, 10-minute hold each). Average BC before and after exposures was determined from sets of five single-fill cystometry infusions. The effect of sacral nerve stimulation was also characterized using implanted InterStim™ systems to deliver stimulation during post-exposure cystometry. Exposure conditions (instilled solutions) and cystometry (with and without neurostimulation) were repeated three times for each animal.

Results: Inspection of the data reveals the following observations: i) variability in the BC change from baseline after RA exposure (decrease vs increase) occurred both within and across animals (BC decreases ranging from 6 to 71%, and BC increases ranging from 6 to 37%); ii) in some trials BC decreased following exposure to control solutions; iii) consistent with previous data in the sheep model, application of bilateral SNM increases bladder capacity.

Conclusions: Intravesical instillation of RA did not have consistent effects on the bladder capacity and therefore the data do not support translation of this RA-induced bladder hyperactivity model into sheep. Based on this study, future efforts should evaluate modifications to the exposure paradigm and alternative chemical irritant formulations toward development of a research model representing OAB for neuromodulation research.

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Title: Expression profile of platelet-derived growth factor receptor α (PDGFRα)-mediated genesets differentiates interstitial cystitis

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Introduction: Platelet-derived growth factor receptor-α (PDGFRα) is a transmembrane tyrosine kinase receptor, which plays a pivotal role in mediating fibrogenesis. Despite the lack of detailed molecular mechanism, several studies have attempted to understand the physiological effect of PDGFRα in human bladder diseases. Here, we provides a novel transcriptomic insight into the impact of PDGFRα in bladder microenvironment and corresponding role in interstitial cystitis (IC) pathogenesis.

Methods: Using RNA-seq techniques, we screened the genome-wide gene expression pattern for the unsorted and sorted PDGFRα immune-positive (PDGFRα+) cells in murine suburothelial and detrusor layers, respectively. We compared the geneset expression pattern between unsorted and PDGFRα+ cells. The differentially expressed genesets were designated as PDGFRα-mediated.

Results: We translationally mapped the PDGFRα-mediated genesets to human IC bladder transcriptomic data and observed a significant overlap between the PDGFRα-mediated genesets and the genesets deregulated in IC. We indicate that i) the PDGFRα-mediated genesets differentiate between IC patients with normal and low bladder capacity and ii) the PDGFRα-mediated genesets differentiate ulcerative IC patients from controls in two independent cohorts, respectively.

Conclusions: Our study suggests a central role of PDGFRα in IC pathogenesis and provides a novel and useful diagnostic method to differentiate IC human subjects. (Support for this project was obtained from NIH/NIDDK R01 DK098388)
PS43

Title: Automated closed-loop stimulation to inhibit neurogenic bladder overactivity

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Introduction: Individuals with spinal cord injury (SCI) usually develop neurogenic detrusor overactivity (NDO), resulting in urinary incontinence, decreased bladder capacity, and reduced quality of life. Electrical stimulation of the genital nerves (GNS) has been shown to inhibit bladder activity to improve bladder capacity and urinary continence in men and women. An automated closed-loop bladder neurostimulation system currently does not exist but could improve efficiency and feasibility of an electrical stimulation approach. We have developed a custom algorithm to identify bladder contractions and trigger stimulation in real time without need for abdominal pressure measurement. The goal of this pilot study was to test the feasibility of automated closed-loop control of GNS using our custom algorithm to identify and inhibit reflex bladder contractions in real time.

Methods: Experiments were conducted in a single session in a urodynamics laboratory in 3 men and 1 woman with SCI and NDO. Each subject completed standard cystometrograms without and with electrical stimulation to the genital nerves. Trial types randomized. Our custom algorithm monitored bladder vesical pressure at 100 samples/second, and controlled when stimulation was turned on and off.

Results: The custom algorithm detected bladder contractions in real time, successfully inhibiting a total of 53 contractions across all 4 subjects. There were 15 false positives, 10 of those occurring in one subject. Four bladder contractions were not successfully identified and/or inhibited, which was associated with bladders near their capacity. It took approximately 4.0±2.6 s for the algorithm to detect the onset of a bladder contraction and trigger stimulation. The algorithm maintained stimulation for approximately 3.5±1.7 s, which was enough to inhibit activity and relieve feelings of urgency. Automated closed-loop stimulation was well-tolerated and subjects reported that algorithm decisions generally matched with their perceptions of bladder activity.

Conclusions: The custom algorithm successfully identified bladder activity with sufficient time to trigger stimulation to reduce urgency and prevent urinary incontinence acutely. Stimulation decisions were made autonomously by the algorithm without human intervention. Closed-loop neuromodulation using only bladder pressure in our custom algorithm may be feasible, but further testing is needed refine and validate this approach to improve bladder control for individuals with SCI and NDO.

Funding Source: SUFU Foundation Study of Neuromodulation
Title: Targeting Fidgetin-like 2 to rescue locomotor and lower urogenital function after spinal cord injury

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Introduction/Objectives: More than a quarter million Americans live with spinal cord injury (SCI) with approximately 12,000 new cases of SCI occurring each year in the United States. Although recovery of locomotion is viewed as a top priority in the public opinion, restoration of urogenital function is regarded by patients as having greater importance. The major impediment for recovering function after SCI is the inability of damaged neurons within the CNS to regrow their axons and overcome inhibitory cues to reinnervate their targets. We recently identified a novel microtubule severing enzyme, termed Fidgetin-like 2 (FL2), which inhibits axonal growth. In vitro, FL2 knockdown has been shown to enhance axonal growth and promote growth through inhibitory substrates. In this study we investigated the extent to which in vivo FL2 knockdown at the site of spinal cord injury rescues the locomotor and urogenital functional in SCI rats.

Methods: Female Wistar rats (300 g) were used in this study and SCI was induced by lateral compression of exposed spinal cord at the T11-T12 level. FL2 depletion at the site of injury was achieved by topical over-dura administration of nanoparticle encapsulated FL2 siRNA (FL2 siRNA-np) at 5 minutes post-injury. Locomotor function was then evaluated by standard “BBB” scoring; bladder function was assessed daily based on total voided urine volume by manual bladder compression and at endpoint by cystometry; erectile function was assessed at endpoint by cavernosometry.

Results: Animals treated with FL2 siRNA-np demonstrated significantly improved motor function at Day 4 after SCI when compared to control SCI rats treated with scrambled siRNA containing nanoparticles. FL2 siRNA-np treated SCI rats also had improved bladder function as evidenced by lower urine expressed volumes as early as 4 days after SCI, and by cystometric findings of increased voided volume and bladder compliance, and decreased micturition frequency, threshold pressure, micturition pressure and spontaneous activity at 2 weeks post-SCI. Moreover, SCI rats treated with FL2 siRNA-np had a trend towards improved erectile response as determined by electrostimulation of the cavernous nerve, resulting in an increased intracavernous pressure (ICP) when compared to control animals.

Conclusions: Strategies devised to knockdown FL2 at the injury site and soon after SCI have the potential to improve locomotor, bladder and erectile function outcomes.

Funding Source(s): New York State SCIRB C31611GG
Title: Extensive sensory decentralization attenuates adenosine triphosphate (ATP) release from bladder intramural nerve endings in canines

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Introduction/Objectives: Utilizing \textit{in vitro} contractile response of bladder smooth muscle strips, we sought to determine the effect of more extensive sensory decentralization on muscle contractility and the effect of reinnervation by nerve transfer.

Methods: Eight canines were decentralized by bilateral transection of all sacral spinal roots; three were sacrificed at 12 months post decentralization (group 1, N=3) and five underwent reinnervation by transfer of the obturator nerve to the anterior vesical branch of the pelvic nerve either immediately (group 2, N=3) or 12-month post decentralization (group 3, N=2). Seven underwent more extensive decentralization by bilateral transection of all sacral spinal roots and L7 dorsal roots; four were sacrificed at 12 months post decentralization (group 4, N=4) and three were reinnervated 12-month post decentralization (group 5, N=3). Hypogastric nerves were transected in all of the aforementioned groups of animals. Sham-operated (N=6) were used as controls. Bladders were harvested and mucosa denuded smooth muscle strips were isolated. Responses to electric field stimulation (EFS) were used to evaluate nerve-evoked contractions. Data was expressed as mean ± SEM. \( P \) values of < 0.05 or less were considered significant. One- and two-way ANOVAs and Bonferroni post-hoc tests were used to determine group differences.

Results: Responses to KCl in strips of all groups were similar and comparable to control, except those of the immediately reinnervated after sacral transection (group 2) which exhibited responses lower than any other group including sham-operated control. Maximum responses to EFS were lower in immediately reinnervated after sacral transection (group 2), greater in 1 year reinnervated after sacral transection (group 3) and similar in all other groups, compared to sham. Calculated half maximal effective frequency (EF50) was significantly lower than sham in all group except group 5 which was not different than sham. The muscarinic antagonist atropine (1 \textmu M) inhibited EFS contractions more in decentralized with all sacral and dorsal L7 roots transected (group 4) and both delayed reinnervation groups (3,5) compared to sham, with no differences in atropine’s effect in the sacral transected (group 1) as well as in their matched immediately reinnervated (group 2). No differences were seen in the EFS induced contractions following purinergic receptor desensitization with 10 \textmu M \( \alpha,\beta \) methylene ATP between groups.

Conclusions: Both purinergic and muscarinic components contributed to nerve mediated contractions. The transection of L7 dorsal roots in addition to sacral roots is required to reduce the purinergic component of nerve mediated contractions due to reduced ATP release from nerve terminals.

Funding Source(s): NIH-NINDS NS070267
Title: Bladder smooth muscle strip contractility studies are reliable up to 48 hours after harvest

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Introduction/Objectives: Smooth muscle contractility studies depend upon the viability of tissue following its harvest. Removing tissue from its in vivo environment stresses a variety of cellular functions, which may alter their response to conditions used during experimentation. We aim to assess changes in smooth muscle contractility immediately (Day 0), 24 hours (Day 1), and 48 hours (Day 2) after collection of female canine bladders.

Methods: Bladders were harvested from animals that underwent the following procedures: Group 1 - one-year decentralization via transection of ventral and dorsal sacral roots and hypogastric nerves, bilaterally (N=3); Group 2 - conditions of Group 1 plus transection of dorsal roots of L7 (N=4); Group 3 - conditions of Group 1 followed by a six-month surgical reinnervation via nerve transfer, bilaterally (N=2); Group 4 - sham-operated control (N=2). Bladders were stored in HTK organ preservation solution at 4°C until studies were conducted. Group 1 was tested on both Day 1 and Day 2 while Groups 2, 3, and 4 were tested on both Day 0 and Day 1. Responses to electric field stimulation (EFS) at varying frequencies, 120 mM potassium chloride (KCl), and 10 mM αβ-methylene ATP (αβmATP) were measured.

Results: 3/8 bladders tested on Day 0 and Day 1 had significantly different responses to electric field stimulation, with measured tension significantly decreasing from Day 0 to Day 1 in one bladder from Group 2 [Day 0: 3.44±0.43 g (n=32 strips) and Day 1: 1.85±0.24 g (n=32), p=0.0014] and significantly increasing in both bladders from Group 3 [Day 0: 2.96±0.34 g (n=32) and Day 1: 4.65±0.60 g (n=30), p=0.022; Day 0: 2.17±0.25 g (n=32) and Day 1: 3.55±0.57 g (n=32), p=0.019]. All bladders tested on Day 1 and Day 2 responded the same across days. Only 1/8 bladders tested on Day 0 and Day 1 from Group 3 had a significantly higher response to KCl [Day 0: 1.65±0.14 g (n=32) and Day 1: 2.48±0.34 g (n=30), p=0.039] while 2/3 bladders tested on Day 1 and Day 2 had a significantly lower response to KCl [Day 1: 1.91±0.18 g (n=16) and Day 2: 1.25±0.21 g (n=16), p=0.0034; Day 1: 2.16±0.37 g (n=16) and Day 2: 1.14±0.14 g (n=16). There were no differences between days in any group when average strip responses per animal were averaged together. Only 1/8 bladders showed a significant increase in αβmATP sensitivity across days [Day 0: 1.07±0.18 g (n=10) and Day 1: 0.61±0.10 g (n=10), p=0.048].

Conclusions: While differences across days were seen within individual bladders, we were able to conclude that the strength of bladder smooth muscle contractility in response to a variety of stimuli do not necessarily decrease with increasing time after collection. Tissue integrity over time should be taken into account to maximize its use, especially when working with a large animal model and resources are limited. Data generated across multiple days can be combined if no significant differences are identified.

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PS47

Title: Lipid Modulation of Pelvic Pain

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Introduction/Objectives: Interstitial cystitis/bladder pain syndrome (IC) is a debilitating condition of chronic pelvic pain with unknown etiology. Our group utilizes a MAPP-endorsed neurogenic cystitis model that recapitulates key aspects of IC, including pelvic pain. We observed previously that B6 and BALB/c mice develop different levels of pain during neurogenic cystitis, suggesting a genetic influence in murine pelvic pain. To identify loci modulating pelvic pain, we performed a QTL analysis of pelvic pain in F2_Cx6 mice and identified Aoah as a locus modulating pain severity.

Methods: Pelvic allodynia as a measure of referred pain in response to infection with PRV Bartha was quantified with von Frey filaments, and mice were genotyped by Illumina MD Linkage Panel. QTL was performed using R/qtl. Aoah expression was evaluated by qRT PCR. Lipids were quantified by HPLC-MS with MRM, and AOAH was localized along the bladder-brain axis by immunofluorescence. Recombinant AOAH was generated in E. coli.

Results: We identified a SNP near Aoah, the gene encoding acyloxyacyl hydrolase. We found that AOAH-deficient mice have elevated pelvic pain responses, and AOAH immunoreactivity was detected along the bladder-brain axis. Pilot metabolomic analyses identified arachidonic acid (AA) as significantly elevated in the sacral spinal cord of AOAH-deficient mice, suggesting AA is a substrate for AOAH. Spinal cord lipidomics revealed increased arachidonic acid-containing phosphatidylcholine in AOAH-deficient mice and concomitant decreased phosphatidylethanolamine, consistent with decreased CoA-independent transferase activity (CoIT), a biochemical activity long postulated to mediate AA homeostasis. Recombinant AOAH protein exhibited CoIT activity that required residues in the putative CoIT domain. In spinal cords, AOAH deficiency was also associated with elevated arachidonic acid and PGE2, and pelvic pain was reduced in AOAH-deficient mice by a PGE2 receptor antagonist.

Conclusions: Together, these findings suggest that AOAH represents a long-sought arachidonic acid CoIT and thereby modulates sensory pathways mediating pelvic pain.

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**Title:** Effect of Parity on Murine Vaginal Wall Elastic Fiber Structure and Mechanical Function

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**Introduction/Objectives:** Pelvic Organ Prolapse (POP) is characterized by the loss of pelvic floor support and descent of the pelvic organs through the vaginal canal. While the definitive etiology of POP is unknown, vaginal birth is a well-established risk factor. Due to ethical issues and limited resources for longitudinal studies, animal models have been leveraged to advance pelvic floor research. Prior work by Downing et al. showed that parity altered elastic fiber structure and structural properties of the rat vaginal wall. Further, mouse models with disrupted elastic fibers readily develop POP, however, the influence of parity on elastic fiber structure in the control mouse model is unknown. Therefore, the objective of this study was to determine the effect of parity on vaginal wall elastic fiber structure and mechanical response. We hypothesized that parous animals will demonstrate altered elastic fiber structure and decreased stiffness.

**Methods:** Vaginal tissue from female nulliparous (n=3) and parous (n=3) C57BL/6 mice at 4-6 months of age (IACUC approved; at estrus) were excised for passive inflation-extension testing. Elastic fiber histological analysis was performed with Hart’s Elastic stain and CT-FIRE software (n=3/group). Student’s t-test were used to determine differences (p<0.05) in mechanical properties and microstructure.

**Results:** Parous vaginal tissue displayed a significantly greater area fraction of elastic fibers (Figure 1B; p<0.01). Further, elastic fiber length was significantly shorter (Figure 1C; p<0.05). Statistical significance was not detected for elastic fiber width and straightness ratio. In addition, compliance, toe modulus, and transition stretch were not statically significant.

**Conclusions:** This study investigated the effect of parity on elastic fiber structure and mechanical response of the murine vaginal wall. Results suggest that pregnancy and vaginal birth may alter elastic fiber structure. Significance in the mechanical response was not detected, which may be a result of the small sample size (n=3). Further biochemical and mechanical analysis are needed to further elucidate the effect of parity on vaginal elastic fibers and contribution to POP.

![Figure 1](image.png)

Figure 1. The effect of parity on vaginal wall (A) mechanical response, (B) elastic fiber area fraction, and (C) elastic fiber length. Parity resulted in an increase in elastic fiber area fraction and decrease in fiber length.

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