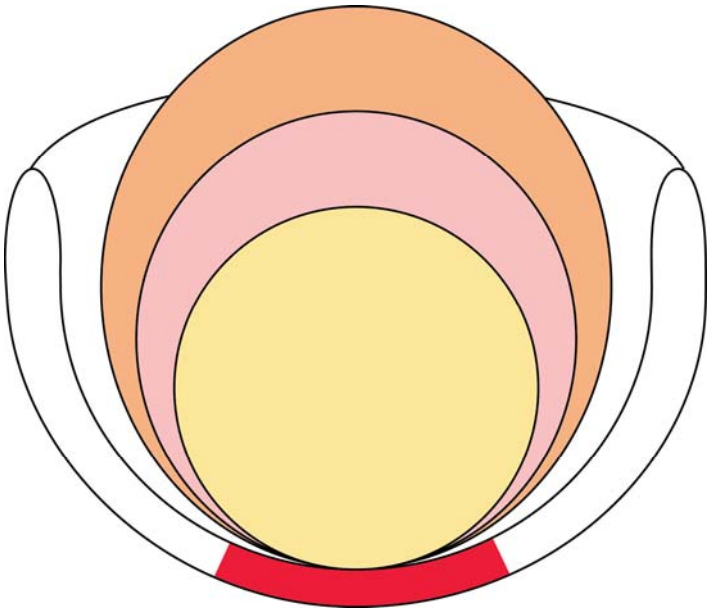
THE SOCIETY FOR PELVIC RESEARCH

SECOND ANNUAL MEETING



MEETING PROGRAM

December 1-3, 2017
Reno, NV

Sponsored by:

A generous donation by the **Glickman Urological & Kidney Institute at Cleveland Clinic**

In Partnership with:

The **International Continence Society** ICS

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, 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.

My Travel ELF
Creating *magical* vacations

The 2017 SPR Abstract Review/Final Meeting Planning Committee

Matthew O. Fraser, PhD
Sean M. Ward, PhD
Margot S. Damaser, PhD
Maryrose P. Sullivan, PhD
Steven D. Abramowitch, PhD
Johanna L. Hannan, PhD
Kelvin P. Davies, PhD
Michael R. Ruggieri, Sr., PhD
Brian M. Balog, PhD Candidate

All those attending and participating in the Second Annual Meeting

Our Mission Statement

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

Our Vision Statement

The Society for Pelvic Research will be the premier professional organization for career basic and translational scientists 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. Other 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 0r 2522.

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

This meeting represents the cumulative efforts of our board and advisors 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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Trainee Affairs Committee Chair
Brian M. Balog, BS, PhD Candidate
Lerner Research Institute at the Cleveland Clinic and The University of Akron

Members at Large
Lori A. Birder, PhD
Professor, Departments of Medicine and Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine

Kelvin P. Davies, PhD
Professor, Departments of Urology and Physiology & Biophysics, Albert Einstein College of Medicine

Sang D. Koh, PhD
Professor, Department of Physiology and Cell Biology, University of Nevada, School of Medicine

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

Program Summary

December 1, 2017

7:00 PM **Trainee Affairs Committee Workshop** - Dr. Johanna L. Hannan, PhD
8:00 PM **Trainee Social Event**

December 2, 2017

7:00 AM **Continental Breakfast**
8:00 AM **Welcome, Opening Remarks, Mission of the SPR** - Matthew O. Fraser, PhD
8:05 AM **Session 1: Novel Therapies/Diagnostics for Pelvic Disorders**
Moderators: Deanna C. Sinex, PhD Candidate and Sang Don Koh, PhD
Key Note Speaker - Dr. Aria F. Olumi, MD
Q&A
Oral Presentations - Abstracts S1A1-S1A4
Q&A
10:10 AM **Break** - F&B
10:25 AM **Session 2: Muscle and Organ Function**
Moderators: Brian M. Balog, PhD Candidate and Maryrose P. Sullivan, PhD
State of the Art Speaker/International Continence Society Lecturer
- Dr. Christopher H. Fry, PhD
Q&A
Oral Presentations - Abstracts S2A5-S2A8
Q&A
12:30 PM **Lunch**
1:30 PM **Session 3: Physiology and Pharmacology I**
Moderators: Michael R. Odom, PhD Candidate and Kelvin P. Davies, PhD
State of the Art Speaker - Dr. Hamid I. Akbarali, PhD
Q&A
Oral Presentations - Abstracts S3A9-S3A12
Q&A
3:35 PM **Break** - F&B
3:50 PM **Session 4: Special Topics I**
Moderators: Katrina M. Knight, PhD and Sean M. Ward, PhD
Special Guest Speaker - Dr. Gene F. Civillico, PhD
Q&A
4:45 PM **Break**
5:00 PM **Poster Session** / **Wine and Cheese Reception**
Moderators: Steven D. Abramowitz, PhD and Michael E. DiSanto, PhD
Poster Presentations - Abstracts PS25-PS35
7:00 PM **Adjourn for the Day**

December 3, 2017

7:00 AM **Continental Breakfast**
8:00 AM **Welcome to Day 2** - Sean M. Ward, PhD
8:05 AM **Session 5: Pelvic Floor**
Moderators: Anna S. Nagle, PhD and Carol A. Podlasek, PhD
Key Note Speaker- Dr. James A. Ashton-Miller, PhD
Q&A
Oral Presentations - Abstracts S5A13-S5A16
Q&A

Program Summary

December 3, 2017

10:10 AM **Break** - F&B

10:25 AM **Session 6: Special Topics II**

Moderators: Shelby A. Powers, Medical/MS Student and Matthew O. Fraser, PhD

Special Guest Speaker – Ms. Sherrie Palm

Q&A

11:05 AM **Session 7: Neuroscience of Pelvic Organs**

Moderators: Trevor C. Hunt, Medical Student and Michael E. DiSanto, PhD

Oral Presentations - Abstracts S7A17-S7A20

Q&A

12:15 PM **Lunch**

1:15 PM **Session 8: Physiology and Pharmacology II**

Moderators: Danielle M. Salvadeo, MD/PhD Student and Johanna L. Hannan, PhD

Oral Presentations - Abstracts S8A21-S8A24

Q&A

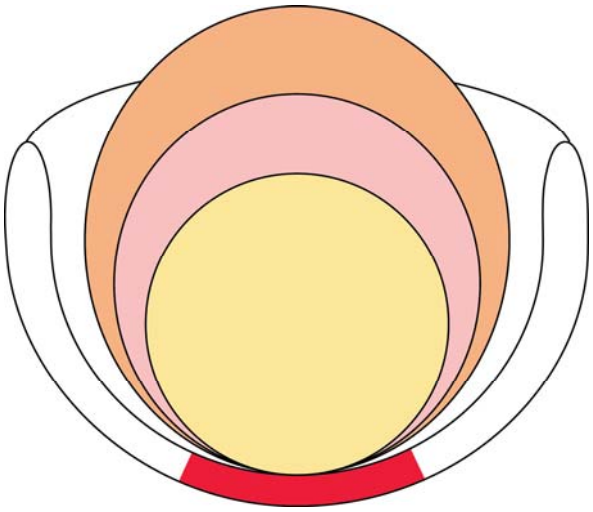
2:25 PM **Break** - F&B

2:40 PM **Awards and Closing Remarks**

Awards: Steven D. Abramowitch, PhD and Michael E. DiSanto, PhD

Closing Remarks: Matthew O. Fraser, PhD

3:15 PM **Meeting Adjourns**



Program in Detail

December 1, 2017

7:00 PM 1:00 **Trainee Affairs Committee Workshop** - *Preparing for Life Post-PhD: Navigating the Job Search* - Dr. Johanna L. Hannan, PhD

8:00 PM 2:00 **Trainee Social Event**

December 2, 2017

7:00 AM 1:00 **Continental Breakfast**

8:00 AM 0:05 **Welcome, Opening Remarks, Mission of the SPR** - Matthew O. Fraser, PhD

Session 1: Novel Therapies/Diagnostics for Pelvic Disorders - Deanna C. Sinex, PhD Candidate and Sang Don Koh, PhD, Moderators

8:05 AM 0:45 **Key Note Address** - *Personalizing Management of BPH* - Dr. Aria F. Olumi, MD
8:50 AM 0:10 Q&A
9:00 AM 0:15 S1A1 - *Measurement of bladder wall micromotion during urodynamics in an anesthetized pig model using M-mode ultrasound* - Anna S. Nagle, PhD
9:15 AM 0:15 S1A2 - *Afferent innervation of the canine bladder protects against urinary tract infections* - Michael R. Ruggieri, PhD
9:30 AM 0:15 S1A3 - *Assessment of Aging Related Changes in the Innervation of the External Anal Sphincter Based on Motor Unit Action Potential Quantitative Analysis* - Nicholas Dias, Graduate Student
9:45 AM 0:15 S1A4 - *Prophylactic Treatment Markedly Improves Bladder Capacity following Pelvic Radiation* - Doreen Chang, MA , Medical Student
10:00 AM 0:10 Panel Q&A

10:10 AM 0:15 **Break** - F&B

Session 2: Muscle and Organ Function - Brian M. Balog, PhD Candidate and Maryrose P. Sullivan, PhD, Moderators

10:25 AM 0:45 **State of the Art/International Continence Society Lecture** - Fibrosis *and the Bladder: Implications for Function* - Dr. Christopher H. Fry, PhD
11:10 AM 0:10 Q&A
11:20 AM 0:15 S2A5 - *Myogenic mechanisms of detrusor overactivity in spinal cord injury* - Haeyeong Lee, PhD
11:35 AM 0:15 S2A6 - *Role of CD38, CD73, and Enpp1 in the degradation of β-nicotinamide adenine dinucleotide (β-NAD) in the murine distal colon* - Violeta Mutafova-Yambolieva, MD, PhD
11:50 AM 0:15 S2A7 - *Cholinergic neurotransmission in the colon is mediated through M3 muscarinic receptors expressed on interstitial cells of Cajal and not smooth muscle cells* - Sung Jin Hwang , PhD
12:05 AM 0:15 S2A8 - *Short-term high fat diet induces increased detrusor contractility independent of mitochondrial dysfunction in C57BL/6N males* - Trevor C. Hunt, Medical Student
12:20 PM 0:10 Panel Q&A

12:30 PM 1:00 **Lunch**

Program in Detail

December 2, 2017

Session 3: Physiology and Pharmacology I - Michael R. Odom, PhD Candidate and Kelvin P. Davies, PhD - Moderators

1:30 PM 0:45 **State of the Art Lecture** - *The Guts of the Opioid Crisis: Role of the Microbiome* - Dr. Hamid I. Akbarali, PhD

2:15 PM 0:10 Q&A

2:25 PM 0:15 S3A9 - *The firing of intracellular Ca2+ waves in mouse urethral smooth muscle relies on a store operated Ca2+ entry pathway to sustain Ca2+ release from ER stores* - Bernard T. Drumm, PhD

2:40 PM 0:15 S3A10 - *NLRP3 Controls Bladder Decompensation during Chronic Bladder Outlet Obstruction* - Francis M. Hughes, Jr, PhD

2:55 PM 0:15 S3A11 - *Elevated hydrostatic pressure stimulates ATP release and mediates activation of the NLRP3 inflammasome via P2X4 in rat urothelial cells* - Cody L. Dunton, Graduate Student

3:10 PM 0:15 S3A12 - *Diabetic changes in function and protein O-GlcNAcylation in visceral organs* - Yifei Xu, PhD

3:25 PM 0:10 Panel Q&A

3:35 PM 0:15 **Break** - F&B

Session 4: Special Topics I - Katrina M. Knight, PhD and Sean M. Ward, PhD - Moderators

3:50 PM 0:45 **Special Guest Lecture** - *NIH SPARC: Mapping the Neural Control of Organ Function* - Dr. Gene F. Civillico, PhD

4:35 PM 0:10 Q&A

4:45 PM 0:15 **Break**

Poster Session / Wine and Cheese Reception

5:00 PM 2:00 **Poster Presentations** - Steven D. Abramowitz, PhD and Michael E. DiSanto, PhD - Moderators

PS25 *Bladder expression of Myosin 5A Isoforms* - Josephine A. Carew, PhD

PS26 *Ca2+-dependent activation of transient receptor potential melastatin-4 channels by sarcoplasmic reticulum inositol trisphosphate receptors in human detrusor* - Aaron Provence, PhD Candidate

PS27 *Direct innervation from lower thoracic and upper lumbar ventral horn to the detrusor is mediated by neuromuscular nicotinic receptors* - Danielle M. Salvadeo, MD/PhD Student

PS28 Moved to Oral Presentation

PS29 *Inflammatory cytokine interleukin 1 beta increases apoptosis in dissociated major pelvic ganglia neurons* - Jennifer C. McMains, Undergraduate Student

PS30 *Lumbar to sacral root rerouting to restore bladder function in a feline spinal cord injury model: urodynamic and retrograde nerve tracing results from a pilot* study – Ornella Lam Van Ba, MD

PS31 *Peripheral nerve neuromodulation of urological function using a distributed sensing and stimulation system* – Lance Zirpel, PhD

PS32 *Comparison of bladder shape between individuals with and without overactive bladder* - Anna S. Nagle, PhD

PS33 *Investigation of Motor Unit Firing Patterns of Bulbospongiosus Muscle in Type II Diabetic Female Patients* - Chuan Zhang, Graduate Student

PS34 *Sonic hedgehog regulation of rhabdosphincter muscle* - Marah Hehemann, MD

Program in Detail

December 2, 2017

PS35 *The Ca2+-activated Cl- channel ANO1 is expressed in mouse urethral interstitial cells of Cajal and contributes to urethral smooth muscle Ca2+ signaling and contractility* - Benjamin E. Rembetski, Undergraduate Student

7:00 PM **Adjourn for the Day**

December 3, 2017

7:00 AM 1:00 **Continental Breakfast**

8:00 AM 0:05 **Welcome to Day 2** - Sean M. Ward, PhD

Session 5: Pelvic Floor - Anna S. Nagle, PhD and Carol A. Podlasek, PhD, Moderators

8:05 AM 0:45 **Key Note Lecture** - *On Pressing Female Pelvic Floor Problems: A Biomechanics Viewpoint* - Dr. James A. Ashton-Miller, PhD

8:50 AM 0:10 Q&A

9:00 AM 0:15 S5A13 - *Changes in Hymenal Ring (HR) Position Resulting from Vaginal Parity* - Deanna C. Sinex, PhD Candidate

9:15 AM 0:15 S5A14 - *Impact of prolapse mesh on vaginal smooth muscle function: a comparison between the rabbit and nonhuman primate* - Katrina M. Knight, PhD

9:30 AM 0:15 S5A15 - A *Simulated Childbirth Injury Model's Recovery is Accelerated with Four Electrical Stimulations a Week* - Brian M. Balog, PhD Candidate

9:45 AM 0:15 S5A16 - *Altered Elastin Homeostasis with Pelvic Organ Prolapse in the Lysyl Oxidase-Like 1 Knockout Mouse Model* - Slater A. Jameson, Medical Student

10:00 AM 0:10 Panel Q&A

10:10 AM 0:15 **Break** - F&B

Session 6: Special Topics II - Shelby A. Powers, Medical/MS Student and Matthew O. Fraser, PhD - Moderators

10:25 AM 0:30 **Special Guest Lecture** – *Comorbidities, Behavior, and Lifestyle: The POP Missing Links* - Ms. Sherrie Palm

10:55 AM 0:10 Q&A

Session 7: Neuroscience of Pelvic Organs - Trevor C. Hunt, Medical Student and Michael E. DiSanto, PhD - Moderators

11:05 AM 0:15 S7A17 - *Elimination of afferent innervation to the bladder disrupts urothelial integrity and modulates NADPH oxidase (Nox)-associated oxidative stress in a canine model of lower spinal cord injury* - Nagat Frara, PhD

11:20 AM 0:15 S7A18 - *Pelvic neuron apoptosis leads to impaired bladder contractility in a rat model of prostatic radiation* - Shelby A. Powers, Medical/MS Student

11:35 AM 0:15 S7A19 - *Sonic hedgehog promotes cavernous nerve regeneration by inducing cavernous nerve sprouting and sprouting potential is reduced with age* - Ryan W. Dobbs, MD

11:50 AM 0:15 S7A20 - *Cavernous nerve crush injury increases apoptosis in a time dependent manner in the entire pelvic plexus* - Marah Hehemann, MD

Program in Detail

December 3, 2016

12:05 AM 0:10 Panel Q&A

12:15 PM 1:00 **Lunch**

Session 8: Physiology and Pharmacology II - Danielle M. Salvadeo, MD/PhD Student and Johanna L. Hannan, PhD - Moderators

1:15 PM 0:15 S8A21 - *In-Vivo hypogastric nerve stimulation and recording during bladder filling in canines* - Ekta Tiwari, Graduate Student

1:30 PM 0:15 S8A22 - *Ex vivo penile vascular function is preserved in male C57Bl6/N mice following a 12 week high fat diet despite diet-induced obesity* - Michael R. Odom, PhD Candidate

1:45 PM 0:15 S8A23 - *Evidence of hyperglycemic memory following diabetic bladder disease* - Yi Wang

2:00 PM 0:15 S8A24 - *Regulation of tone in the IAS by phasic events: role of L-type calcium channels and stretch* - Caroline A. Cobine, PhD

2:15 PM 0:10 Panel Q&A

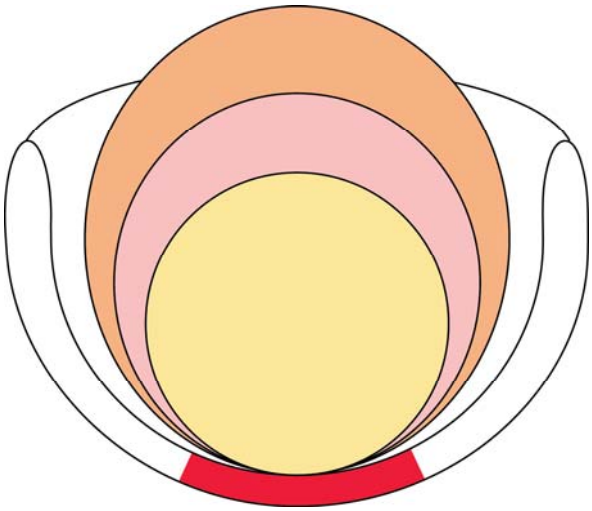
2:25 PM 0:15 **Break** - F&B

Awards and Closing Remarks

2:40 PM 0:30 **Awards:** Steven D. Abramowitch, PhD and Michael E. DiSanto, PhD

3:10 PM 0:05 **Closing Remarks:** Matthew O. Fraser, PhD

3:15 PM **Meeting Adjourns**



Key Note Speaker

Dr. Aria F. Olumi, MD

Department of Urology
Massachusetts General Hospital
Boston, Massachusetts

Dr. Aria Olumi, MD is a Professor of Surgery/Urology at Harvard Medical School, and the Program Director for the Urology Residency Program and Director of Research for Department of Urology for Massachusetts General Hospital. He also serves as the Chair of Research Council for the American Urological Association. As a physician scientist at Massachusetts General Hospital & Harvard Medical School, his clinical activities are focused on urologic oncology and surgical management of kidney, bladder and prostate cancer. Previously, Dr. Olumi served as the director of the Combined Harvard Urologic Oncology Fellowship Program, an alliance between Massachusetts General Hospital and Brigham and Women's Hospital.

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"Personalizing Management of BPH"

My NIH funded laboratory is focused on providing personalized care for prostatic diseases. We have learned that the 5-alpha reductase 2 gene, an important gene that is responsible for normal prostatic development and growth, and a target of the commonly used 5-alpha reductase inhibitors (finasteride or dutasteride), is not expressed in 30% of adult men. We have defined the epigenetic mechanism that leads to variable expression of the gene in adult prostates. Our findings have broad implications that can explain the variable growth patterns of the prostate gland and the reason for resistance to the therapeutic effects of 5-alpha reductase inhibitors."

Key Note Speaker

James A. Ashton-Miller, PhD

Biomechanics Research Laboratory & Pelvic Floor Research Group
Department of Mechanical Engineering
University of Michigan, Ann Arbor, Michigan

Dr. James Ashton-Miller joined the University of Michigan in 1983 where he directs the Biomechanics Research Laboratory. He, his students and collaborators use theoretical and experimental approaches to understand the biomechanics of unintentional injuries so they and their sequelae can be better prevented. He has authored over 250 scientific articles (h-index: 61), six patents and graduated 30 doctoral students. He is a cofounder of the Pelvic Floor Research Group at the University of Michigan which has won numerous awards for its research over the past 25 years. He is a past-President of the American Society of Biomechanics (ASB), a fellow of ASME, AIMBE, ASB and GSA, and winner of the 2009 ASB Giovanni Borelli award, the 2015 ASME H.R. Lissner Medal, and the 2016 Kappa Delta award from the Orthopedic Research Society and American Academy of Orthopedic Surgeons. Dr. Ashton-Miller received his B.Sc (Hons) from Newcastle University, U.K., his MSME from M.I.T., U.S.A. and Ph.D. from the University of Oslo, Norway.

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"On Pressing Female Pelvic Floor Problems: A Biomechanics Viewpoint"

Over the past decades there has been good progress towards a better understanding of the functional anatomy of the female pelvic floor in health and disease. However, women continue to sustain injuries during vaginal birth; those injuries lead to later sequelae that include urinary incontinence and pelvic organ prolapse. After briefly reviewing these, we shall discuss the research that is needed over the next years for us to do better - prevent these problems in the first place. A successful vaginal birth is one in which the fetal head is pressed through the pelvic floor during the second stage of labor in three hours or less without damaging the pubovisceral muscles. They form the last part of the birth canal to be engaged by the fetal head and we know from computer models that these muscles have to stretch to over three times their original length in order to accommodate the fetal head. How they do that remains a mystery (and would make a good doctoral dissertation topic). Is it actually the muscles that stretch, is it the perineal body into which they insert, or is it both? Our research (Tracy et al, J. Biomech. Eng. 2016) does provide a framework for predicting how well the fetal head will fit through the birth canal before the second stage of labor starts and when there will be problems because of cephalo-pubovisceral muscle disproportion. But we need to understand how and where the birth canal ripens in preparation for vaginal birth, the hormones that are involved, which tissues ripen, what exactly happens at the molecular and cellular levels, why the ripening can be inadequate in terms of the required changes in elastic or viscous behavior of the tissues, what material property values represent healthy behavior and when they are abnormal, what can be done to assist inadequate ripening, and so on. There are therefore opportunities for meaningful research at every scale, from the molecular level through the organ level to the whole body level.

State of the Art Speaker
International Continence Society Lecturer

Dr. Christopher H. Fry, PhD

School of Physiology, Pharmacology & Neuroscience
University of Bristol, Bristol, United Kingdom

Dr. Christopher Fry, PhD is Professor of Applied Physiology and Chairman of Department of Physiology, Pharmacology & Neuroscience, University of Bristol, UK. He has authored 165 scientific articles in the fields of Urology and Cardiology. He has served as the Chair of the Board of Trustees for the Physiological Society, a member of the Court of Examiners for and is a Fellow of the Royal College of Surgeons of England, and serves as an external evaluator for biomedical sciences training, for the Portuguese Education Ministry. He received a PhD in Physiology in 1975 and a DSc in Physiology in 1991.

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"Fibrosis and the Bladder: Implications for Function"

The bladder wall generates an increase of wall tension for voiding, and when relaxed contributes to a high bladder compliance to allow effective filling. Apart from discrete cellular components of the bladder wall, such as detrusor smooth muscle, an important functional fraction is an extracellular matrix (EM) of proteins including collagen and elastin. The biomechanical properties and the relative quantity of the EM have significant effects on the overall physical properties of the bladder wall. During detrusor contraction force generated by muscle cells is transmitted through this EM, and in doing so EM will impart its own influence on the magnitude and temporal profile of tissue contraction. Moreover, when detrusor muscle is relaxed changes to passive wall tension during filling will be importantly affected by the biomechanical properties of the EM itself and contribute to bladder compliance. In addition, cells contributing to EM production can also generate agents that have more direct contractile consequences.

Many lower urinary tract pathologies are characterised by increased deposition of EM – fibrosis. The affected tissue is thickened which may be regarded as an exaggerated wound healing response. In the lower urinary tract clinical conditions associated with fibrosis include: bladder outflow obstruction, detrusor overactivity, congenital anomalies and chronic inflammation; as well as a result of external agents such radiation and certain drugs. A principal source of EM is the fibroblast and transformed phenotypes including myofibroblasts. A key consideration is to understand the external agents that influence these cells to generate EM and also to consider how fibroblasts themselves act in an autocrine or paracrine fashion through their secretion of profibrotic factors.

I will describe: the deposition of EM in clinical conditions affecting the lower urinary tract; how fibrosis affects the contractile properties of muscular tissues of the lower urinary tract; how external factors modulate the function of fibroblasts; how novel advances may be used to regulate EM deposition.

State of the Art Speaker

Dr. Hamid I. Akbarali, PhD

Department of Pharmacology and Toxicology
Virginia Commonwealth University, Richmond, Virginia

Dr. Hamid Akbarali, PhD is Haag Professor of Pharmacology, Virginia Commonwealth University and Vice Chair of the Department of Pharmacology and Toxicology. He is also the Director of the VCU Initiative for Maximizing Student Development (IMSD) program. Dr Akbarali obtained his PhD from University of Newfoundland, Canada. He was Assistant Professor at Harvard Medical School and Professor of Physiology at University of Oklahoma prior to moving to Virginia in 2005.

"The Guts of the Opioid Crisis: Role of the Microbiome"

Opioid-related mortality has reached epidemic proportions in the USA resulting in staggering costs to the nation both economically and socially. Opioids remain the mainstay for the treatment of moderate to severe pain, however the development of tolerance and the paradoxical hyperalgesia to the effect of these excellent pain relievers are considered as major factors resulting in dose escalation and to overdose. Understanding the basis for the tolerance to physiological effects of opioids is therefore essential in mitigating this crisis. Our studies are focused on the mechanisms of tolerance to the analgesic and constipating effects of opioids. Recent studies from our laboratory have shown that chronic opioids induce gut microbial dysbiosis resulting in a "leaky" gut and subsequent inflammation in the colon through cytokine release from enteric glia. Current concepts of the gut-brain interaction in analgesic tolerance and the role of peripheral mechanisms emanating from the gut will be presented.

Special Guest Speaker

Dr. Gene F. Civillico, PhD

National Institute of Health Common Fund, SPARC
National Institutes of Health (NIH), Bethesda, Maryland

Dr. Gene Civillico, PhD is Program Manager for Stimulating Peripheral Activity to Relieve Conditions (SPARC), a program of the NIH Common Fund. Dr. Civillico earned his Ph.D. in neuroscience at the University of Pennsylvania School of Medicine, studying the neurophysiology of sensory integration in the rodent cerebral cortex in vivo. Following postdoctoral training in cerebellar physiology and two-photon microscopy, Dr. Civillico joined Otsuka Pharmaceutical Company, where he led a small early-stage discovery team. Dr. Civillico moved to FDA's Center for Devices and Radiological Health in 2011, where he developed a research portfolio addressing roadblocks to the translation of neuroengineering and rehabilitation research concepts into clinical solutions. He joined the Office of the NIH Director in 2016 to lead SPARC, and maintains a guest research affiliation with the medical device laboratories at FDA/CDRH.

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"NIH SPARC: Mapping the Neural Control of Organ Function"

The NIH SPARC program funds collaborative teams to develop and use the latest anatomical, physiological, and computational methods to map the peripheral innervation of select organs. The program seeks to catalyze the discoveries that will support the next generation of peripheral neuromodulation devices and protocols. SPARC project teams are managed as a consortium in which data sharing and collaboration are required, and teams have access to centralized data and simulation-hosting resources being developed for use by all. SPARC's structure allows for a unique degree of flexibility to respond to new scientific or technological developments. Currently, the SPARC consortium includes several projects of potential interest to the Society for Pelvic Research, including anatomical and functional mapping of bladder innervation in humans and animal models, as well as electrode and bladder sensor development.

SPARC is a program of the NIH Common Fund, managed by the Office of Strategic Coordination in the Office of the NIH Director. The Common Fund supports catalytic, transformative programs that cut across the missions of multiple NIH Institutes and Centers. These programs are intended to change paradigms, develop innovative tools and technologies, and/or provide fundamental foundations for research that can be used by the broad biomedical research community.

Special Guest Speaker

Sherrie Palm

Founder/CEO/Executive Director
Association for Pelvic Organ Prolapse Support (APOPS)
Mukwonago, Wisconsin

Sherrie Palm is the Founder/CEO/Executive Director of Association for Pelvic Organ Prolapse Support (APOPS), a pelvic organ prolapse (POP) Key Opinion Leader, author of award winning book Pelvic Organ Prolapse: The Silent Epidemic, a speaker on multiple aspects of pelvic organ prolapse quality of life impact, and an international women's pelvic health advocate. Sherrie's points of focus are generating global POP awareness, developing guidance and support structures for women navigating POP, and bridge building within POP healthcare, research, academia, industry, and policy sectors toward the evolution of POP directives. Additional information about APOPS, pelvic organ prolapse, or Ms. Palm's book or speaking presentations is available on the website below.

http://www.pelvicorganprolapsesupport.org

"Comorbidities, Behavior, and Lifestyle: The POP Missing Links"

Layout of my presentation is 3 sections, with a focus throughout on the value of research to advance POP arena. Three sections are an intro to APOPS (who we are, what we are doing), value of research to clarify reality vs abundant misconceptions in POP, and POP risk factors not currently well recognized or being researched. For lack of time, I am focusing on 3 layers: Ehlers-Danlos-hypermobility/POP intersect; Fitness training, running, jogging/POP intersect; and value of estrogen above and beyond current common standard practice.

The POP Missing Links: Pelvic organ prolapse is a diverse condition with multiple causal factors. Comorbid conditions, behaviors, and lifestyle choices typically advance POP and/or increase symptomology. This session will review common POP causes not currently well recognized or explored, with a focus on generating scientific exploration.

ICS 2018 PHILADELPHIA

28-31 August 2018

Leading Continence Research and Education

Call for Abstracts:
1 March - 3 April 2018

International Continence Society
48th Annual Meeting

www.ics.org/2018

S1A1

Title: Measurement of bladder wall micromotion during urodynamics in an anesthetized pig model using M-mode ultrasound

Authors: Anna S. Nagle[1], Zachary E. Cullingsworth[1], Uzoma A. Anele[2], Charles R. Blocher[2], Adam P. Klausner[2], and John E. Speich[1]

Affiliations: [1]Department of Mechanical & Nuclear Engineering, Virginia Commonwealth University, Richmond, VA. [2]Department of Surgery, Virginia Commonwealth University, Richmond, VA.

Introduction/Objectives: Bladder wall micromotion caused by low amplitude rhythmic contractions (LARC) of the bladder wall may play a key role in producing bladder sensation and urgency. A non-invasive method to detect micromotion would be an important step in accessing conditions such as detrusor overactivity. The aim of this study was to develop an anesthetized porcine model to compare LARC quantified from urodynamic pressures with micromotion quantified with non-invasive M(motion)-mode ultrasound cine loops of the bladder wall.

Methods: Female pigs anesthetized with urethane underwent urodynamic studies with a fill rate of 50 ml/min. At bladder volumes of 250 ml and 500 ml, filling was paused and the pig was removed from the ventilator for 60 sec so that image data could be obtained without respiratory or filling motion. A correlation-based motion tracking algorithm was implemented in MATLAB to measure the width of the bladder wall over time in user-selected regions of interest (ROIs). The changes in bladder wall width were compared to changes in vesical pressure (Pves).

Results: **Figure 1** shows a frame of an ultrasound M-mode cine loop in which bladder wall thicknesses were tracked in two ROIs (**A**) and the changes in bladder width within those ROIs overlaid on Pves (**B**). The correlation between anterior and posterior bladder wall width compared with Pves were 0.61 and 0.62 respectively ($p<0.001$).

Conclusions: An anesthetized pig model exhibiting LARC was developed. A moderate correlation was found between bladder wall width and Pves. This shows proof of concept for measuring bladder wall micromotion non-invasively with ultrasound.

Funding Source(s): This research was funded by the SUFU-Cogentix Medical OAB grant.

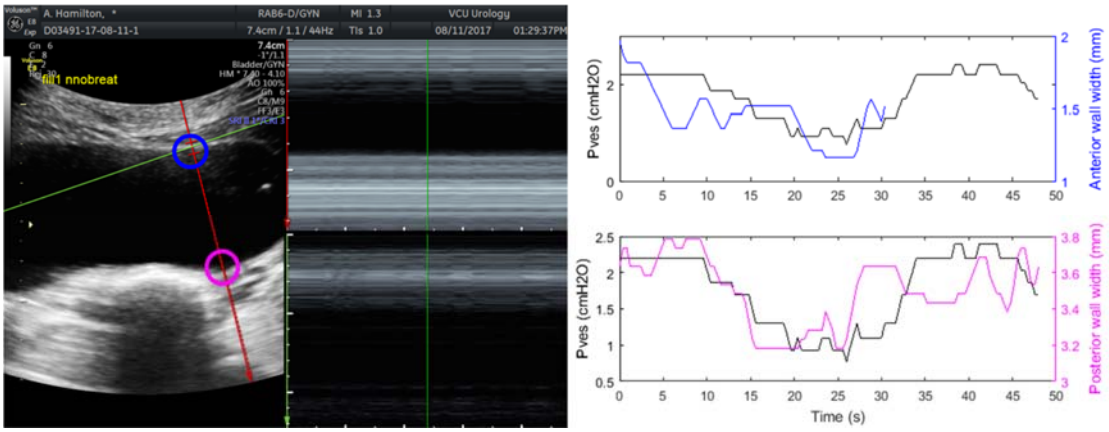

Fig 1. A) Ultrasound image of a pig bladder with anterior (blue) and posterior (magenta) ROIs indicated. **B**) Changes in anterior (top) and posterior (bottom) bladder wall width overlaid on Pves.

S1A2

Title: Afferent innervation of the canine bladder protects against urinary tract infections.

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Introduction/Objectives: We previously observed urination and defecation postures in animals decentralized by transection of all roots (dorsal and ventral) below L7 indicating sensory recovery. We determined whether more extensive decentralization surgeries would eliminate these urination and defecation postures and whether these animals might be more susceptible to urinary tract infections (UTI).

Methods: Three groups of progressively more extensive pelvic decentralization surgeries were studied including bilateral transection of 1> all spinal roots caudal to L7 and the hypogastric nerves (N=6), 2> all roots caudal to L7, the hypogastric nerves and the L7 dorsal roots (N=8) and 3> all roots caudal to L7, the hypogastric nerves and both the L6 and L7 dorsal roots (N=8). Urination and defecation postures were monitored with video surveillance cameras over the housing cages. UTIs were monitored by urine Multistix® test strip assays and culture and sensitivity of mid-stream urine specimens by a commercial clinical microbiology laboratory.

Results: All of the animals in group 1 with intact L6 and L7 afferent bladder innervation showed micturition postures but none had evidence of UTIs up to one year after decentralization surgery. In group 2 with the additional L7 dorsal root transections, 4 consistently showed micturition postures up to 12 months postoperatively with no evidence of UTIs. The remaining 4 showed the appearance of micturition postures during periods when UTIs were confirmed with urine culture and Multistix® urine assays positive for blood, leukocytes and nitrates. These postures disappeared with successful antibiotic clearance of the UTIs. UTIs were confirmed in 6 of the 8 animals in group 3 with both L6 and L7 dorsal root transections but none showed micturition postures up to 2 months after decentralization. Three animals in this group including the remaining 2 with no evidence of UTI were euthanized one month after decentralization due to inability to regain complete hind limb function in one and hind limb self-mutilation in the other 2.

Conclusions: Because no evidence of UTIs were observed in our previous series of animals decentralized by transection of all roots below L7 but intact hypogastric nerves as well as in group 1 of the present study with the additional hypogastric nerve transections, lack of sympathetic bladder innervation has no apparent effect on susceptibility to UTIs. A greater incidence of UTIs (50% of animals) was observed with the additional elimination of bladder afferents by L7 dorsal root transections. Even greater incidence (75%) was found with both L7 and L6 dorsal root transection indicating that eliminating afferent innervation of the bladder predisposes the bladder to UTI. We are currently pursuing the hypothesis that the afferent innervation of the urothelium may maintain the integrity of its antibacterial defense mechanisms perhaps through reactive oxygen species generating enzymes such as NADPH oxidases.

Funding Source(s): NIH RO1NS 070267

S1A3

Title: Assessment of Aging Related Changes in the Innervation of the External Anal Sphincter Based on Motor Unit Action Potential Quantitative Analysis

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Introduction/Objectives: Prior research suggests that muscles undergo denervation and compensatory reinnervation during aging. Reinnervation after denervation increases the size of motor unit action potentials (MUAP). The progression of denervation and compensatory reinnervation of the external anal sphincter (EAS) has been studied with single fiber electromyography (EMG) and other intramuscular EMG techniques. Current intramuscular EMG techniques are painful, invasive and some require extensive expertise to operate. This project aims to assess the denervation and subsequent re-innervation in the external anal sphincter associated with aging using a non-invasive intra-rectal high density surface EMG probe.

Methods: Young (n=6, 35.7±4.9 yr) and elderly (n=7, 67.5±11.8 yr) subjects were recruited to participate in this study. A 64-Channel (8x8) high density surface EMG (HDsEMG) probe was inserted into the rectal space of the subject. EMG signals were acquired during a series of five maximum voluntary contractions of the external anal sphincter. The resulting HDsEMG recordings were decomposed into MUAP spike trains using our developed KmCKC decomposition technique. The MUAP amplitude was defined as the amplitude of the most negative peak that is present at the MUAP innervation zone.

Results: Table 1 shows the average results of MUAP quantitative analysis. The increase in MUAP amplitude was found to be close to significant by a two-sample t-test (p<.058).

Table 1 summarizes the results of MUAP analysis.

	Age [Years]	Amplitude(*) [μV]	Decomposition Yield [MUs]
Young (n=6)	35.7±4.9	50.35±16.18	7.67±3.5
Elderly (n=7)	67.5±11.8	74.16±23.07	7.71±1.11

Conclusion: This study is the first to examine EAS denervation and subsequent reinnervation during aging using advanced noninvasive HDsEMG techniques. Our results suggest that HDsEMG paired with KmCKC decomposition is a suitable tool to detect changes in MUAP amplitude associated with aging.

Funding Sources: This work was supported by NIH DK082644

S1A4

Title: Prophylactic Treatment Markedly Improves Bladder Capacity following Pelvic Radiation

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Introduction/Objectives: Radiation cystitis (RC) occurs frequently following radiotherapy to the pelvis for treatment of malignancy. In some patients the late toxicity of radiation cystitis has devastating health outcomes. The acute phase of RC may be due to loss of urothelial barrier function. We hypothesize that the acute phase contributes or is directly responsible for the late phase. Utilizing a previously established animal model we trialed a novel therapeutic strategy to prevent the acute phase of RC.

Methods: Using our previously described radiation cystitis model in the rat, 7 female Sprague Dawley rats received pre-radiation treatment with triamcinolone acetonide, 200 mg/ml in 50% dimethyl sulfoxide (DMSO) solution, 0.5 ml instilled into the bladder via the indwelling bladder catheter, while 10 served as controls with normal saline instillation. All test animals received single dose bladder irradiation with 20 Gy using a CT image guided irradiator. Bladder function was evaluated on a weekly basis for all animals for 9 weeks by conscious restrained cystometry using sequential infusions of normal saline, 300 and 500 mM KCl (≥ 60 minutes for each infusate; 0.10 ml/min flow rate). Cystometric functional and total bladder capacities (FBC, TBC) were recorded. Data were analyzed using 2−Way ANOVA and linear regression models with post-hoc testing.

Results: Prophylactic treatment resulted in marked improved in mean TBC from week 3 onward as follows: up to 64% greater following saline challenge ($P=0.0008$), up to 72% greater following 300KCl challenge ($P=0.0021$), and up to 91% greater following 500 KCl challenge ($P=0.0001$). Similarly mean FBC was consistently higher from 3 week onward as follows: by up to 38% greater following saline challenge ($P=0.0034$), up to 37% greater following 300KCl challenge ($P=0.003$), up to 49% greater following 500 KCl challenge ($P<0.0001$).

Conclusions: These data demonstrate that prophylactic treatment with triamcinolone acetonide in DMSO solution results in improved cystometric profile in irradiated rats when compared to controls. These results may be translatable into future therapies to prevent RC in patients undergoing pelvic radiotherapy.

Funding Source(s): Duke Urology

S2A5

Title: Myogenic Mechanisms of Detrusor Overactivity in Spinal Cord Injury

Authors: Haeyeong Lee PhD*, Byoung Koh BS, Robert Corrigan BS, Lauren Peri BS, Kenton Sanders PhD and Sang Don Koh MD PhD

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Introduction/Objectives: The bladder has the unique capability of maintaining low muscle excitability during filling. We discovered and characterized an entirely novel control mechanism that regulates detrusor excitability. PDGFRα^{+} cells supply a powerful inhibitory mechanism to the bladder during filling. This mechanism is composed of the following molecular and functional components: 1) PDGFRα^{+} cells display strong expression of SK3 channels (*Kcnn3*). 2) Hyperpolarization due to activation of SK channels in PDGFRα^{+} cells exerts a membrane potential-stabilizing effect on detrusor muscles. 3) Expression of TRPV4 channels provides a stretch-dependent source for Ca^{2+} entry and activation of SK channels. 4) Genetic deactivation of TRPV4 and SK3 channels result in bladder overactivity. The hypothesis of this present study is the loss or defects in PDGFRα^{+} cells or in key molecular components of the inhibitory regulation provided by PDGFRα^{+} cells in spinal cord injury (SCI) leads to detrusor dysfunction and development of an overactive phenotype.

Methods: SCI was induced by complete compression of T13-L1 spinal cord. Experiments were performed on 24 hr, 48 hr and 72 hrs after surgery. We employed molecular approaches (RNAseq, qPCR and protein studies) and *ex vivo* cystometry.

Results: In *ex vivo* cystometry, SCI bladder revealed an increase in the amplitude and frequency of transient contractions (TCs; relevant to non-voiding contractions in *in vivo* cystometry) during filling. TCs increased in SCI bladders. Effects of a SK blocker (apamin) and a SK channel activator (SKA31) on TCs were reduced in SCI mice compared to control suggesting downregulation of SK channels in SCI bladder. qPCR, immunohistochemistry and immunoblot showed the loss of PDGFRα and downregulation of SK channels. RNAseq and qPCR data showed the apoptosis-related geneset score was significantly increased in SCI detrusor muscles.

Conclusions: These findings support that loss or downregulation of PDGFRα^{+} cells and SK channels in SCI detrusors might involve the development of detrusor overactivity from SCI.

Funding Source(s): Supported by NIDDK, RO1 DK098388

S2A6

Title: Role of CD38, CD73, and Enpp1 in the degradation of **β**-nicotinamide adenine dinucleotide (**β**-NAD) in the murine distal colon

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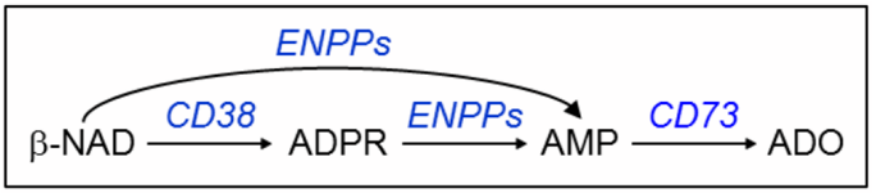
Introduction/Objectives: **β**-NAD and its primary metabolite ADP-ribose (ADPR) are enteric inhibitory motor neurotransmitters in the large intestine of different species, including mice and primates. Degradation of released neurotransmitter is a common way of terminating neurotransmitter action at the neuroeffector junction. CD38, CD73, and ecto-nucleotide pyrophosphatases (ENPPs) contribute to the extracellular metabolism of β-NAD (see Figure), but the relative functions of these enzymes in the colon have not been examined. The goal of the present study was to investigate the role of CD38, CD73, and Enpp1 in the degradation of β-NAD in the colon and to determine the contribution of smooth muscle cells (SMC), interstitial cells of Cajal (ICC), and platelet-derived growth factor α+ (PDGFRα+) cells, also called the SIP syncytium, in β-NAD degradation.



Methods: Colonic *tunica muscularis* was isolated from C57BL/6 (wildtype, WT) mice, *Cd38*$^{-/-}$ mice, *Nt5e (Cd73)*$^{-/-}$ mice, *Enpp1*$^{-/-}$ mice, and *Cd38*$^{-/-}$*/Cd73*$^{-/-}$ mice. SMC, ICC and PDGFRα+ cells were isolated from colons of *smMHC*$^{Cre\text{-}egfp}$ mice, Kit$^{+/copGFP}$ mice, and *PDGFRα*$^{egfp/+}$ mice, respectively, by FACS sorting. Tissue segments or cells were loaded in a small-volume (50-200 µl) superfusion system and superfused with the substrate 1,*N*6-etheno-NAD (eNAD, 0.2 µM) for 0.5-2 min. Decrease in substrate and increase in product was evaluated with a HPLC technique with fluorescence detection as a measure of enzyme activity.

Results: In WT mouse colon, eNAD produced eADPR, eAMP, and eADO. In *Cd38*$^{-/-}$ mouse colon, eNAD formed eAMP and eADO, but not eADPR. In *Cd73*$^{-/-}$ mouse colon, eNAD produced eADPR and eAMP, but no eADO. In *Cd38*$^{-/-}$*/Cd73*$^{-/-}$ mouse colon, eNAD produced no eADPR and negligible amounts of eADO whereas eAMP was increased. In *Enpp1*$^{-/-}$ mouse colon, eNAD produced more eADPR and less eAMP and eADO. Therefore, in the mouse colon, β-NAD is degraded to ADPR by CD38, ADPR is degraded to AMP by ENPP1, and AMP is degraded to ADO by CD73. eNAD produced eAMP and eADO primarily in SMC and to a lesser extent in PDGFRα+ cells and not in ICC, whereas accumulation of eADPR was not detected in any of the SIP cells.

Conclusions: CD38 participates in β-NAD degradation in whole tissue, but it is likely present in cells that are different from the SIP cells (e.g., neurons) in the colon. CD73 that degrades β-NAD through AMP to ADO is primarily located on SMC and to a lesser degree on cells, but not on ICC. ICC do not appear to be the place for β-NAD degradation in the murine colon. Metabolism of β-NAD by multiple enzymes likely contributes to the complex nature of purinergic signaling in the colon. Levels of extracellular β-NAD and metabolites can be altered by targeting specific metabolic pathways in the colon.

Funding Source(s): NIH grant DK 41315

S2A7

Title: Cholinergic neurotransmission in the colon is mediated through M_3 muscarinic receptors expressed on interstitial cells of Cajal and not smooth muscle cells.

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Introduction: The excitability of gastrointestinal (GI) smooth muscles is regulated by several different classes of interstitial cells (interstitial cells of Cajal (ICC) and PDGFRα^+ cells that are electrically coupled to neighboring smooth muscle cells (SMCs). Thus, 'myogenic' activity results from the integrated behavior of the SMC/ICC/ PDGFRα(+) cell (**SIP**) syncytium. Inputs from excitatory and inhibitory motor neurons are required to produce the complex motor patterns of the GI tract. Motor neurons innervate these cell types in the **SIP** syncytium, and receptors, second messenger pathways, and ion channels in these cells mediate postjunctional responses that regulate motor activity. Cholinergic neurotransmission in GI muscles from several species has long been thought to be dependent upon activation of a non-selective cation conductance in smooth muscle cells and the molecular candidates for mediating cholinergic excitation have been reported to be mediated by M_2 and M_3 muscarinic receptors,[1,2] via the transient receptor protein channels *Trpc4* and *Trpc6* in SMCs.[3] However, it has been shown that cholinergic motor responses in several regions of the gastrointestinal tract occur via ICC, in particular intramuscular ICC that form close anatomical relationships with enteric nerve terminals.

Objectives: We sought to determine the muscarinic receptor(s) and the cell type(s) that are responsible for cholinergic motor responses in the colon.

Methods: Cre-LoxP recombinase technology was utilized to determine the role of M_3 muscarinic receptors. We utilized "floxed" M_3 receptor gene mice (M_3-LoxP mice) and crossed them separately with *smMHC/Cre* (Mike Kotlikoff, Cornell University, NY) and *c-Kit*$^{CreERT2Ejb1/+}$ (*c-Kit-Cre*) mice (Deiter Sauer, Technical University, Munich) to produce cell-specific knock-down of M_3 receptors when treated with tamoxifen (i.e. SMC and Kit$^+$ ICC specific). Intracellular microelectrode recordings and isometric force measurements were performed to determine changes in post-junctional excitatory neural responses to nerve stimulation using electrical field stimulation (EFS).

Results: Knock down of M_3 receptors in Kit$^+$ ICC using Cre/Lox P technology caused a marked reduction in muscarinic-sensitive cholinergic excitatory responses in the colon. Using a similar strategy to knock down M_3 receptors in smooth muscle cells did not produce a loss in excitatory motor responses in colonic muscles in response to EFS, compared to wildtype controls and tamoxifen injected controls.

Conclusions: These data suggest that M_3 receptors expressed in ICC are the biological transducer of cholinergic excitatory responses in ICC and not smooth muscle cells is important for cholinergic excitatory post-junctional neural responses in colonic muscles.

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S2A8

Title: Short-term high fat diet induces increased detrusor contractility independent of mitochondrial dysfunction in C57BL/6N males

Authors: Trevor C. Hunt[1]*, Michael D. Tarpey[1,2], Michael R. Odom[1], Elena S. Pak[1], Espen E. Spangenburg[1,2], Johanna L. Hannan[1,2]

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Introduction/Objectives: High fat diet (HFD) is associated with obesity and can lead to cardiac and skeletal mitochondrial dysfunction. However, the contribution of mitochondria to the development of bladder dysfunction has not been examined. Our aims are to characterize mitochondrial function in the urothelium and detrusor and compare to detrusor contractility following 6 weeks of HFD. We hypothesize that 6 weeks of HFD will increase mitochondrial respiration, detrusor contractility, and voiding.

Methods: Male C57BL/6N mice (10wks) were fed control (10% fat) or HFD (45% fat) for 6 weeks. Body weight, body composition via MRI, and glucose tolerance were measured. Bladder physiology was assessed at 3 and 6 weeks of diet via void spot assays. High-resolution respirometry measured mitochondrial oxygen consumption at complexes I, II, and IV in isolated urothelium and detrusor samples (n=7/group). Mitochondrial derived hydrogen peroxide (H_2O_2) production was also assessed. In a separate group of mice, detrusor strips with and without urothelium were placed in myographs and detrusor contractility was assessed by bolus administration of high potassium as well as concentration response curves to carbachol and electric field stimulation (EFS) (n=6/group).

Results: Mice fed a HFD for 6 weeks exhibited increased body weight and body fat % ($p<0.001$) in addition to elevated fasted blood glucose levels ($p<0.01$). Voiding frequency and volume did not change with HFD, although bladder weight did increase ($p<0.05$). Oxygen consumption rates in control bladders were doubled in the urothelium at complex I, complex IV, and complexes I+II compared to the detrusor ($p<0.05$). Additionally, the urothelium produced significantly greater amounts of H_2O_2 ($p<0.05$). This indicates that the urothelium may have greater mitochondrial content compared to detrusor smooth muscle. Overall, oxygen consumption rates and H_2O_2 production in the urothelium and detrusor from 6 week HFD mice were unchanged compared to age matched controls. Following 6 weeks of HFD, contractile responses to high potassium, carbachol, and EFS were unchanged in detrusor strips with urothelium. In denuded strips, 6 weeks of HFD increased contractility with high potassium, high concentrations of carbachol, and high frequency EFS stimulation ($p<0.05$).

Conclusions: This study is the first to examine mitochondrial respiration in urothelium and detrusor smooth muscle. The urothelium has greater rates of mitochondrial respiration and ability to generate H_2O_2 compared to the detrusor smooth muscle. After 6 weeks of HFD, there was no change in bladder mitochondrial respiration; however, increased smooth muscle contractility was evident. Thus, in male C57BL/6N mice fed a HFD for 6 weeks, increased bladder contractility is occurring independently of aberrations in mitochondrial function.

Funding Source(s): BSOM Summer Scholars Research Award; BSOM startup funds.

S3A9

Title: The firing of intracellular Ca^{2+} waves in mouse urethral smooth muscle relies on a store operated Ca^{2+} entry pathway to sustain Ca^{2+} release from ER stores

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Introduction/Objectives: During bladder filling, urethral smooth muscle cells (USMC) generate myogenic tone and thus malfunctions in USMC contractility can lead to incontinence. Non-invasive and long-term successful treatments for incontinence are lacking and thus there is a clear need to improve our understanding of USMC physiology to identify cellular targets in incontinence disorders. Currently, there is a lack of imaging studies on USMC motor activity *in situ* and the specific nature of Ca^{2+} signaling in these cells is unknown. We sought to use Ca^{2+} imaging to investigate and characterize spontaneous activity in USMC *in situ*.

Methods: In our study, we characterized murine USMC activity *in situ*, utilizing Ca^{2+} imaging with a genetically encoded Ca^{2+} reporter, GCaMP3, activated by a specific smooth muscle promoter via an inducible Cre -Lox P system. Ca^{2+} signals were recorded *in situ* using an upright fluorescent microscope. Enzymatically dispersed USMC from SMC-eGFP^{+} mice were collected via fluorescence activated cell sorting for qPCR analysis. Functional experiments on urethral contractility were carried out using ring preparations from C57 wild-type mice measuring isometric tension.

Results: USMC fired spontaneous intracellular Ca^{2+} waves at a mean frequency of 51.1 ± 2.3 min^{-1} (n=31, c=301). Ca^{2+} waves were increased in frequency by the α1 agonist phenylephrine (PE, 10 μM), purinergic agonists ATP (10 μM) and ADP (10 μM) and almost abolished by the nitric oxide (NO) donor DEA-NONATE (10 μM). Both Ca^{2+} influx and Ca^{2+} release from intracellular stores contributed to Ca^{2+} waves as Ca^{2+} free solution and blocking the sarcoplasmic Ca^{2+}-ATPase with thapsigargin (10 μM) abolished all activity. Intracellular Ca^{2+} release involved cooperation between ryanodine receptors (RyRs) and inositol tri-phosphate receptors (IP_3Rs) as both tetracaine (100 μM) and 2-APB (10 μM) reduced Ca^{2+} waves. qPCR revealed that USMC expressed the ER Ca^{2+} release channels IP_3R1,2 and RyR1-3. Ca^{2+} waves were insensitive to Cav1.2 channel modulators nifedipine (1 μM), nicardipine (1 μM), FPL (1 μM), and the T-type Ca^{2+} channel blockers NNC 55-0396 (1 μM) and TTA-A2 (1 μM). Ca^{2+} wave frequency was reduced by the store operated Ca^{2+} entry (SOCE) blocker SKF 96365 (10 μM). qPCR showed that USMC express the SOCE channels Orai1-3 and Ca^{2+} waves were reduced by a specific inhibitor of Orai1, GSK-7975A (1 μM). GSK-7975A (3-10 μM) also reduced PE induced contractions of USM.

Conclusions: In conclusion, USMC fire Ca^{2+} waves *in situ*, which are modulated by adrenergic, purinergic and nitrergic inputs. Ca^{2+} waves rely on intracellular Ca^{2+} release via RyRs and IP_3Rs and Ca^{2+} release is sustained by a Cav1.2 and T-type independent Ca^{2+} influx mechanism involving SOCE via Orai1.

Funding Source(s): R01 DK-091336, P01 DK41315

S3A10

Title: NLRP3 Controls Bladder Decompensation during Chronic Bladder Outlet Obstruction

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Introduction/Objectives: Bladder outlet obstruction (BOO) is most often the result of benign prostatic hyperplasia and may lead to progressive voiding dysfunction. Acutely, BOO can create an overactive (irritative) phenotype with increased frequency and urgency coupled with decreased voiding volume (VV). However, at this stage the bladder can typically compensate for the obstruction and expel all fluid. As time progresses, in some men there is an increase in post-void residual volume (PVR) as voiding efficiency drops and the bladder becomes decompensated. We have previously shown in the rat that the NLRP3 inflammasome in the urothelia plays a central role in acute BOO (12 days) where it triggers inflammation, increased voiding frequency and decreased VV. In the present study we have extended the experiment to examine bladder function after chronic BOO (42 days) to determine if NLRP3 plays a role in the decompensation that is often observed clinically at this stage.

Methods: Female rats were divided into 4 groups: control, sham, BOO or BOO+gly (glyburide; an NLRP3 inhibitor). BOO was created by inserting a 1 mm transurethral catheter, tying a suture around the urethra, and removing the catheter. Glyburide was provided with a 50 mg, 21 day release pellet (s.c.) that was replaced after 21 days. At 35 days suprapubic tubes were implanted and at 42 days urodynamics was performed. PVR was assessed immediately after the final micturition.

Results: Voiding pressures were increased and flow rates decreased in BOO and BOO+gly groups, demonstrating physical obstruction of the urethra. No difference in frequency or VV were detected among groups. PVRs were greatly increased in BOO rats while BOO+gly rats were not different than controls. This was also reflected in bladder capacity (VV+PVR). There was a dramatic decrease in voiding efficiency (VV/capacity), indicative of decompensation, in the chronic BOO rats. Efficiency was completely restored to control values by glyburide treatment.

Conclusions: The results suggest a critical role of the NLRP3 inflammasome in mediating functional bladder decompensation during chronic BOO. Recent studies have implicated NLRP3 and IL-1β, the product of the active inflammasome, in the onset of fibrosis and denervation in the bladder in the acute setting. Thus, it is likely that controlling these processes throughout the chronic time period contributes to the prevention of decompensation that we measured. Clinically, the development of NLRP3 inhibitors may be very useful to protect against bladder decompensation over time in men with BOO.

Funding Source(s): NIDDK: R01DK103534

S3A11

Title: Elevated hydrostatic pressure stimulates ATP release and mediates activation of the NLRP3 inflammasome via $P2X_4$ in rat urothelial cells

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Introduction/Objectives: Bladder outlet obstruction (BOO) results from a multitude of etiologies and is projected to affect approximately 1.1 billion men and women by 2018.[4] Bladders of these patients are typically characterized by inflammation, fibrosis, and reduced tissue compliance leading to overactive bladder symptoms.[1] Since the lumen of the obstructed bladder is subjected to chronically elevated storage and voiding pressures, we hypothesize that pathological pressures resulting from BOO initiates a cascade of events that includes release of ATP by urothelial cells, the formation of the NLRP3 inflammasome, and caspase-1 activation. Previous research in our lab demonstrated that ATP release by primary rat urothelial cells increased when they were exposed to elevated pressure (10-15cmH_2O) *in vitro.*[3,5] In the present study, we examined the mechanisms of pressure-induced purinergic signaling and caspase-1 activation in a rat urothelial cell line *in vitro*.

Methods: Using a custom built pressure system[5], rat urothelial cell line MYP3 cells ($1.2x10^6$ cells/well) were exposed to pressure conditions that represent both pathological storage and voiding pressures: 15 cmH_2O 60 min and 40 cmH_2O 1 min. Cells maintained under atmospheric pressure served as a control. After exposure to pressure, the ATP concentration in the supernatant was measured using a commercially available kit (Life Technologies). Cells were then lysed and intracellular caspase-1 activity was measured using an established method.[2] Pressure experiments (40 cmH_2O 1 min) were repeated in the presence of antagonists for purinergic receptors (20 μM 5-BDBD for $P2X_4$, 10 μM A-438079 for $P2X_7$ and 25 μM PPADS for non-specific) or a cocktail of drugs to deplete extracellular ATP (10 ng/mL Brefeldin A, 100 μM Probenecid, and 1 U/mL Apyrase) to determine the mechanism for pressure-induced caspase-1 activation. In addition, ATP-dose response of MYP3 cells for intracellular caspase-1 levels were quantified in the presence or absence of the antagonists.

Results: Exposure of MYP3 cells to hydrostatic pressure for 60 min at 15 cmH_2O and 1 min at 40 cmH_2O both resulted in an increase (>2 fold) in extracellular ATP levels as well as intracellular caspase-1 activity (>1.3 fold) compared to the 0 cmH_2O control. Treatment of MYP3 cells with $P2X_7$ antagonist and non-specific purinergic antagonist upon exposure to 40 cmH_2O for 1 min resulted in a decrease in extracellular ATP levels and caspase-1 activity compared to non-treated cells, while treatment with $P2X_4$ antagonist had no effect on extracellular ATP levels, but decreased caspase-1 activity back to basal levels. MYP3 cells exhibited an ATP dose dependent caspase-1 response that was knocked-down upon treated with $P2X_4$ antagonist and non-specific purinergic antagonist, while $P2X_7$ antagonist had no effect on caspase-1 levels. Lastly, treatment of cells with a drug cocktail that reduced extracellular ATP levels resulted in no increase in caspase-1 levels upon exposure to 40 cmH_2O for 1 min.

Conclusions: The significant increase in ATP release after exposure to 15 cmH_2O for 60 min and 40 cmH_2O for 1 min indicates that MYP3 cells are sensitive to changes in hydrostatic pressure that may be encountered in obstructed bladders. The acute caspase-1 response after 1-minute exposure to pressure suggests that high-pressure voiding may be an important trigger of the NLRP3 inflammasome in BOO. Reduction in pressure-induced ATP release in the presence of $P2X_7$ antagonist indicates the role of $P2X_7$ in amplifying extracellular ATP concentration via ATP mediated ATP release. In contrast, reduction in pressure- and ATP-induced caspase-1 activation following treatment of MYP3 cells with $P2X_4$ antagonist indicates that $P2X_4$ is an important mediator of NLRP3 inflammasome activation. The results of the present *in vitro* study suggest that elevated intravesical pressure associated with BOO is a triggering signal for purinergic activation of the NLRP3 inflammasome pathway, and provide a potential drug target for the treatment of BOO related bladder inflammation.

Funding Source(s): NIH (R01DK103534, P20GM103444), NSF (1264579)

S3A12

Title: Similar changes in the molecular profile and function of visceral organs from an animal model of diabetes

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Introduction/Objectives: Type-2 diabetes (T2D) is associated with a multitude of gastrointestinal and lower urinary tract symptoms, but the mechanisms are not well understood. The chronic hyperglycemia of T2D increases the extent of protein O-GlcNAcylation (modification of serine/threonine residues by the moiety, N-acetyl-glucosamine) and interferes with insulin responsiveness by altering the activity of key proteins in the insulin signaling pathway. This study compared smooth muscle function and protein O-GlcNAcylation patterns in two visceral organs, stomach and bladder, of wild type and db/db mice between 10 and 40 weeks of age.

Methods: Gastric fundus and bladder were obtained from db/db and control mice, the mucosa was removed, and longitudinal smooth muscle strips from each organ were mounted in tissue baths containing Kreb's solution for isometric tension studies. Smooth muscle contractions or relaxations in response to either nerve- or agonist-induced stimulations were generated and compared between diabetic and control animals. The O-GlcNAcylation patterns of parallel tissue samples were assessed by western blotting.

Results: Electrical field stimulation (EFS)-induced relaxations in db/db mice were comparable to control up to 20 wks of age, but were attenuated after 30 wks. The off-response to EFS was also reduced in db/db fundus at 30 wks. Compared to controls however, relaxation in the fundus induced by a NO donor was not reduced in db/db mice at 20 and 40 wks. EFS-induced contraction of bladder smooth muscle tissue was lower in db/db mice than in control tissue at 10 and 20 wks, but no differences were detected between groups at 30 or 40 wks. Post-junctional bladder contractions elicited by CCh and KCl were comparable between groups. Antibody to serine/threonine-O-GlcNAc identified a group of proteins that were more extensively modified in db/db than in control mice, a pattern that was observed in both gastric and bladder tissue. Akt, a kinase whose activation downstream of insulin signaling has been linked to vesicle exocytosis, was identified as a highly O-GlcNAcylated protein in diabetic tissue.

Conclusions: The decreased neurogenic responses in fundic relaxations and bladder contractions in db/db tissues together with the unchanged post-junctional inhibitory or excitatory responses suggest that neurotransmission is impaired in T2D. Increased Akt O-GlcNAcylation potentially explains the impaired neurotransmission observed in the visceral organs of db/db mice.

Funding Source(s): Department of Veteran Affairs, Research Service, Washington DC.

S5A13

Title: Changes in Hymenal Ring (HR) Position Resulting from Vaginal Parity

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Introduction/Objectives: Pregnancy and vaginal childbirth result in significant anatomic changes within the female pelvis that may eventually manifest as pelvic organ prolapse. The pelvic organ prolapse quantification exam utilizes clinical measurements made with respect to the hymenal ring (HR) to describe the type and degree of prolapse in women. Ideally, an anatomical landmark used as a reference point should remain fixed to allow for repeatable and reliable measurements and valid comparisons. We therefore asked, "Does the position of the HR change in the anterior/posterior direction within the pelvis of vaginally parous versus nulliparous women".

Methods: T2 Fast Spin Echo patient scans of vaginally parous (n = 6) and nulliparous women (n = 8), aged 20-49, were acquired through IRB# PRO15060009. Sagittal, Axial, and Coronal plane MRI volumes were co-registered in 3DSlicer (http://www.slicer.org) using bony landmarks to account for patient movement between scans. The distance from the most posterior inferior aspect of the pubic symphysis to the most inferior portion of the anterior and posterior border of the HR were measured in the sagittal plane. These values were then subtracted to also provide a measure of the diameter of the HR. Two-tailed t-tests were conducted to assess differences in measured values between nulliparous and parous women.

Results: The anterior and posterior borders of the HR were closer to the pubic symphysis in nulliparous vs parous women (14.1±1.8mm vs 17.4±3.6mm, p=0.05 and 15.8±1.6mm vs 23.7±5.1mm, p=0.01 respectively). The diameter of the HR was also significantly smaller for nulliparous women than parous women (1.8±0.7mm vs 6.4±4mm, p=0.04 respectively).

Conclusions: The position of the HR shifts posteriorly and increases in diameter following vaginal delivery, suggesting that the HR in fact is not a fixed landmark. Additional work is necessary to see if the HR shifts further in aging patients and/or patients with pelvic organ prolapse that could result in an under appreciation of the clinical presentation.

Funding Source(s): Provost Development Fund, University of Pittsburgh

S5A14

Title: Impact of prolapse mesh on vaginal smooth muscle function: a comparison between the rabbit and nonhuman primate

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Introduction/Objectives: Currently, the nonhuman primate (NHP), Rhesus Macaque, serves as the primary model for evaluating prolapse mesh. The NHP is an expensive model and a limited resource. Thus, a less costly, large animal model is needed. The objective of this study was to compare the impact of prolapse mesh on vaginal smooth muscle (VSM) function in the rabbit as compared to our historical NHP data. Previously we have shown that prolapse mesh has a substantial negative impact on the VSM in the NHP.

Methods: Restorelle (Coloplast), prolapse mesh, was implanted on the vagina of 5 New Zealand rabbits via an abdominal sacrocolpopexy for 3 months. Four rabbits served as sham (no mesh implanted). At 3 months, the mesh-vagina complexes (MVCs) were excised, cut into 7 mm x 2 mm strips, and then submerged in an organ bath containing Krebs solution. Contraction of the smooth muscle was induced by stimulating the tissue with 120 mM KCl. The resulting contractile force was recorded. These data were compared to our historical data following the same protocol in the NHP (N=8 Restorelle, N = 8 sham). Mann-Whitney U and Independent T tests were used to compare the contractile forces between and within species, when appropriate.

Results: The contractile force of the rabbit sham and mesh implanted VSM was 726% and 720% higher than the NHP sham and mesh implanted VSM, $p = 0.007$ and $p = 0.003$, respectively. Interestingly, implanting Restorelle resulted in a 45.9% ($p = 0.014$) decrease in the contractile force of the rabbit VSM, and this result is similar to the 45.5% ($p = 0.027$) decrease observed with the implantation of Restorelle in the NHP.

Conclusions: Similar relative decreases in VSM function following Restorelle implantation in the rabbit and NHP suggest the rabbit may be a useful model for mesh implantation studies on VSM.

Funding Source(s): Department of Defense, Magee Womens Research Institute, DOD 195979

S5A15

Title: A Simulated Childbirth Injury Model's Recovery is Accelerated with Four Electrical Stimulations a Week.

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Introduction/Objectives: Stress Urinary Incontinence is the leakage of urine and its primary risk factor is childbirth, which is associated with pudendal nerve (PN) dysfunction. During childbirth, the PN and the muscle it innervates, the external urethral sphincter (EUS), are injured. A dual nerve and muscle injury model has shown a dysregulation of BDNF, delaying nerve regeneration resulting in delayed functional recovery. Electrical stimulation (ES) has been shown to accelerate nerve regeneration and functional recovery. Twice weekly stimulation decreases functional recovery time after a simulated childbirth injury model. We hypothesized more frequent ES per week will have greater recovery after a simulated childbirth injury model than fewer ES per week.

Methods: Rats underwent either sham injury or dual nerve and muscle injury, PN crush (PNC) and vaginal distension (VD). Wire electrodes were placed in all PNC + VD animals and half the sham injured animals. Sham animals that received electrodes received sham stimulation (SI + SS). PNC + VD animals were divided into three treatment groups: 1) receiving sham stimulation (DI + SS), 2) receiving ES 4 times a week (DI + 4ES), and 3) receiving daily ES (DI + DES). ES consisted of 1 hour of ES at 20 Hz, 0.1ms, 0.3 mA under isoflurane anesthesia for 2 weeks, while sham stimulation was 1 hour of isoflurane anesthesia. Four weeks after the injury leak point pressure (LPP) with simultaneous EUS electromyography (EUS EMG) and pudendal nerve sensory branch Potential (PNSBP) were recorded. ANOVA followed by a Student-Newman-Keuls post hoc test was used to determine significant difference between groups ($p<0.05$). Data is shown as mean ± standard error of the mean.

Results: LPP decreased significantly after DI + SS (19.0 ± 2.43 cm H_20) compared to sham injury with no implanted electrodes (SI + NI) (40.6 ± 3.45 cm H_20) or SI + SS (39.9 ± 2.31 cm H_20), but LPP was not significantly decreased after DI + 4ES (39.9 ± 3.0 cm H_20). EUS EMG was significantly decreased after DI + SS (3.3 ± 1.77 mV) and DI + DES (1.2 ± 0.52 mV) compared to SI + NI (13.5 ± 3.79 mV) or SI + SS (13.7 ± 3.97 mV), but not significantly decreased in DI + 4ES (5.8 ± 1.75 mV).

Conclusions: While ES did not improve PNSBP recovery at both frequencies, stimulation 4 times per week showed full LPP recovery and improved EUS EMG recovery ~~best~~. Daily stimulation appears to be too frequent and maybe causing a re-injury to the nerve, preventing full recovery of LPP.

Funding Source(s): VA Merit A1262-R and the RR&D Service of the VA

S5A16

Title: Altered Elastin Homeostasis with Pelvic Organ Prolapse in the Lysyl Oxidase-Like 1 Knockout Mouse Model

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Introduction/Objectives: Pelvic organ prolapse (POP) decreases quality of life for many women, but its pathophysiology is poorly understood. We have previously shown that Lysyl oxidase-like 1 knockout (*Loxl1 KO*) mice reliably prolapse with age and increased parity, similar to women. Both this model and clinical studies indicate that altered elastin metabolism in pelvic floor tissues plays a role in POP manifestation, although it is unknown if this is a cause or effect. Using *Loxl1* KO mice, we investigated the effects of genetic absence of *Loxl1*, vaginal parity, and presence of POP on the expression of genes and concentration of proteins key to the production and regulation of elastic matrix.

Methods: Six *Loxl1* KO multiparous non-prolapsed (MNP) and five age-matched prolapsed (MP) mice were used. Three age-matched virgin nulliparous (N) *LOXL1* KO and six virgin age-matched wild type (WT) mice were studied as controls. Cultured cells isolated from vaginal explants of mice were assayed with Fastin for elastin, as well as RT-PCR and Western blot for expression of genes and proteins important for elastin homeostasis, including collagen 1A, matrix metalloproteinases 2 (MMP-2) & 9 (MMP-9), tissue inhibitors MMPs 1 (TIMP-1) and 4 (TIMP-4), lysyl oxidase (LOX), transforming growth factor β1 (TFG-β) and bone morphogenic protein 1 (BMP-1). Protein concentration ratios determined the balance between MMPs and TIMPs. Data analysis assessed effects of *Loxl1* absence, delivery, and prolapse on the resultant data. Repeated measures mixed regression methods were used to compare results between groups. Pairwise comparisons were adjusted for multiple comparisons using Tukey-Kramer or Bonferroni corrections with $p<0.05$ indicating a statistically significant difference.

Results: Elastin significantly decreased with absence of *Loxl1.* In contrast, elastin increased with parity ($p < 0.001$), but not with POP, suggesting changes to elastin structure rather than amount in prolapsed mice. Cells from prolapsed mice significantly decreased production of MMP-2 ($p < 0.05$) and increased production of TIMP-4 ($p < 0.05$), suggesting poor post-partum elastin turnover, resulting in accumulation of damaged elastin fibers that can lead to abnormal tropoelastin deposition. Gene expression data generally corroborated that of protein concentration.

Conclusions: We hypothesize that the failure of cells from *Loxl1 KO* mice to produce sufficient amounts of MMP-2 to initiate the post-partum remodeling process results in the accumulation of damaged elastic fibers that serve as an improper scaffold for future tropoelastin deposition and eventually to development of POP. POP may thus, be the result of an inability to initiate the molecular mechanisms necessary to clear and replace damaged elastic matrix in pelvic floor tissues after vaginal delivery.

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S7A17

Title: Elimination of afferent innervation to the bladder disrupts urothelial integrity and modulates NADPH oxidase (Nox)-associated oxidative stress in a canine model of lower spinal cord injury

Authors: Nagat Frara[1], Danielle M. Salvadeo[1], Ekta Tiwari[3], Arielle Roberts[1], Michael Mazzei[2], Alan S. Braverman[1], Mary F. Barbe[1], Changhao Wu[5] and Michael R. Ruggieri[1,3,4].

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Introduction/Objectives: The urothelium contributes to bladder function by communicating with the central nervous system via afferent nerves. We observed recurring urinary tract infections (UTIs) in extensively decentralized animals via elimination of afferent tracts to the bladder. As the highest producer of reactive oxygen species (ROS) and NADPH oxidases (Nox) in the body, we hypothesize that afferent innervation of the urothelium preserves its biological processes, including defense against microorganisms through ROS-generating Nox enzymes.

Methods: Two groups of female hound dogs were used: sham-operated control (n=3) or 12-month decentralized (n=2). Decentralization was performed through bilateral transection of all spinal roots caudal to L7, including the dorsal root of L7 and the hypogastric nerves. Following euthanasia, fresh bladder tissue was isolated and utilized for biochemical assays and immunohistochemistry. Superoxide production in bladder tissue was detected and quantified using Dihydroethidium (DHE) fluorescence and Lucigenin-enhanced chemiluminescence, respectively. Confocal microscopy was used to visualize the DHE-stained sections. Tiron, a superoxide scavenger, will be used to verify the detection of superoxide. Data was analyzed using an unpaired t-test.

Results: Confocal images of DHE fluorescence in bladder sections revealed intracellular superoxide production with the highest density in the urothelium. Lucigenin assay showed that NADPH-dependent superoxide production levels in bladder urothelium of decentralized animals were decreased by 2½-fold, compared to that of sham animals. Tiron eliminated intracellular superoxide signal determined by DHE fluorescence and attenuated superoxide production determined by lucigenin assay in bladder tissue of both decentralized and sham animals.

Conclusions: Blocking of endogenous ROS production confirms their existence in the bladder urothelium of both groups. Animals that showed occurrence of UTIs due to eliminated afferent innervation to their bladders subsequently exhibited lower levels of urothelial superoxide production, compared to controls. It is reasonable to speculate that the afferent–urothelial interactions are essential to the regulation of normal bladder function and that its disruption can impair epithelial function in the decentralized animals. It is also possible that the increased occurrence of UTIs in the absence of afferent innervation is related to the loss of cellular stress response and tolerance capacity mediated by ROS. Our preliminary results demonstrate that the mechanism by which urothelial dysfunction after decentralization of bladders creates susceptibility to UTIs may involve Nox-driven superoxide redox processes.

Funding Source(s): NIH RO1NS 070267

S7A18

Title: Pelvic neuron apoptosis leads to impaired bladder contractility in a rat model of prostatic radiation

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Introduction/Objectives: Men diagnosed with prostate cancer are often treated with radiation therapy (RT). Although the goal of treatment is to eradicate malignant cells, healthy tissues, such as nearby neural structures, also receive radiation. Urinary incontinence is a sequelae of RT even as direct dose to the bladder is minimized. RT induced changes to the nerves controlling bladder function have not been explored. The aim of this study was to determine how prostate RT affects major pelvic ganglia (MPG) neuron growth and apoptosis, as well as bladder smooth muscle function in a rat model of prostatic RT.

Methods: Male 8-week-old Sprague-Dawley rats, underwent pelvic CT imaging and RT planning for delivery of a single 25 Gy radiation fraction via conformal arc or sham treatment. Rats were euthanized and MPG and bladders excised 2 and 10 weeks following RT (n=10/grp). MPG neurons were dissociated and cultured. After 72h, cultures were stained with beta-tubulin and examined for axon length, branching, and apoptosis with TUNEL assay. Bladder muscle strips were cut, one denuded of the urothelium, and both mounted in tissue baths. Concentration response curves to carbachol, and electrical field stimulated (EFS) contraction were assessed.

Results: 2 weeks post-RT, neuron cultures from irradiated MPGs showed less branching ($p<0.05$) with increased neurite length ($p<0.01$). After 10 weeks, neurites also exhibited decreased branching ($p<0.0001$), but with reduced length ($p<0.0001$). The relative number of apoptotic neurons more than doubled at both 2 and 10 weeks (2wk - RT: 5.0%; Sham: 2.1%; 10wk - RT: 9.7%; Sham: 4.0%; $p<0.001$). Bladder to body weight was significantly increased 2 weeks post-RT ($p<0.05$). Carbachol and EFS mediated contraction was significantly decreased at higher concentrations and frequencies in denuded strips 2 weeks post-RT ($p<0.05$). Bladder to body weight was unchanged between RT and age matched controls 10 weeks post-RT. Carbachol contractions were similar between 10 week groups; however, EFS mediated contraction was increased at high frequencies in 10 week RT in both intact and denuded bladder strips ($p<0.05$). Ongoing histological studies will assess if smooth muscle, collagen deposition and innervation of the bladder has changed with RT.

Conclusions: This work is the first to examine prostatic RT effects on the MPG and downstream bladder smooth muscle function. Pelvic neurons demonstrated RT induced apoptosis at early time points that persisted or increased at 10 weeks post-RT. Early post-RT, the bladder demonstrated decreased detrusor contractile responses to carbachol. After 10 weeks the contractile responses to carbachol had normalized while EFS contraction had increased in both intact and denuded strips. These data indicate that preservation of the pelvic ganglia during prostatic RT is critical to maintaining bladder physiology.

Funding Source(s): BSOM seed grant

S7A19

Title: Sonic hedgehog promotes cavernous nerve regeneration by inducing cavernous nerve sprouting and sprouting potential is reduced with age.

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Introduction/Objectives: Sonic hedgehog (SHH) protein delivered by nanoparticle based peptide amphiphile (PA) nanofiber hydrogel to the cavernous nerve at the time of crush injury (prostatectomy model), is neuroprotective, promotes cavernous nerve (CN) regeneration, and return of erectile function, in a rat model. Little is known about the mechanism of how SHH promotes CN regeneration. We hypothesize that SHH promotes sprouting of pelvic ganglia and CN neurons that innervate the penis, in order to enhance regeneration. We examine this hypothesis, and the effects of aging on sprouting potential, in an in vitro organ culture model.

Methods: The caudal portion of the pelvic ganglia and CN were dissected from adult Sprague Dawley rats (n=63), and aged (n=10) rats, and were grown on Matrigel in growth factor reduced medium for three to five days. Pelvic ganglia/CN were exposed to Affi-Gel beads containing: 1.) SHH protein, 2.) 5e1 and cyclopamine SHH inhibitors, and 3.) SHH protein delivered by PA. Additional pelvic ganglia/CN tissue underwent CN crush and were exposed to SHH protein or mouse serum albumin protein (control) by PA in vivo for 4 days with an additional 4 days in culture. Sprouting was evaluated for number of sprouts (growth cones/mm) and their length, and by immunohistochemical analysis for sprouting markers (GAP43 and nNOS).

Results: Sprouting of neurons in the pelvic ganglia and CN were increased with SHH treatment. Sprouts were more abundant, longer in length, and had increased branching, in comparison to controls. Sprouting was even further enhanced in CN injured nerves with SHH treatment. Sprouting did not occur in the presence of either SHH inhibitor. SHH induced sprouting even when not delivered to the CN until 4 days after injury. Sprouts continued to grow in organ culture once initiated with SHH PA in vivo. Localization of SHH delivery makes a difference in sprouting potential. Sprouts formed in response to SHH treatment stained strongly for nNOS and GAP43. Sprouting was reduced in normal aged rats and after CN crush.

Conclusions: SHH PA treatment promotes CN regeneration by enhancing sprouting of pelvic ganglia and CN neurons. Sprouting is reduced in aged rats, which impacts

S7A20

Title: Cavernous nerve crush injury increases apoptosis in a time dependent manner in the entire pelvic plexus.

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Introduction/Objectives: Seventy-two percent of prostatectomy patients develop stress urinary incontinence (SUI) in the first week after surgery, and individuals who do not recover within 6 months, generally do no regain function without intervention (44%). Preoperative erectile function predicts post-prostatectomy continence and SUI recovery correlates with neurovascular bundle sparing, suggesting the importance of maintaining neural innervation. The hypogastric (HYG) and pelvic nerves (PN) control bladder neck and bladder contraction/relaxation. We hypothesize that the HYG and PN may be injured during prostatectomy, in a similar manner to cavernous nerve (CN) injury, and thus contribute to post-prostatectomy SUI development. We will examine HYG, PN and CN architecture and signaling in normal pelvic ganglia and in a rat prostatectomy model.

Methods: The pelvic plexus in normal (n=9), sham (n=8) and CN crushed (n=20) adult Sprague Dawley rats was examined for apoptotic index and sonic hedgehog (SHH) pathway signaling by TUNEL, and immunohistochemical analysis for cleaved caspase-3 (apoptosis indicator), -8, -9, SHH and its receptor Patched.

Results: Cleaved caspase-3 was present in normal pelvic plexus and increased in a time dependent manner in all nerves of the pelvic plexus when the CN was crushed. Caspase 3 cleaved was identified primarily in glial cells, rather than neurons. Caspase 9 increased in glial cells of all nerves of the pelvic plexus, while very little caspase 8 was observed. SHH was abundant in neurons and glia of the CN, PN, HYG and ANC, while PTCH1 was identified only in neurons.

Conclusions: Interruption of CN innervation, as occurs in the majority of prostatectomy patients, results in induction of apoptosis in other regions of the pelvic plexus, thus affecting continence. Apoptosis occurred primarily through the intrinsic pathway in response to CN injury. Identification of HYG and PN contribution to SUI, and involvement of the SHH pathway, identifies novel treatment avenues for intervention.

Funding Source(s): NIH/NIDDK DK101536

S8A21

Title: In-Vivo hypogastric nerve stimulation and recording during bladder filling in canines.

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Introduction/Objectives: We aimed to stimulate and record hypogastric nerve activity during bladder filling in normal intact bladders for eventual application to monitoring sensory reinnervation of the bladder following nerve transfer.

Methods: Female mixed-breed hounds (N=13) weighing 20-25 Kg and 6-8 months of age were used. Maximum increased detrusor pressures were determined following electrical stimulation (3-5mA, 20Hz) of hypogastric nerves (N=13 canines) under isoflurane inhalation anesthesia. A total of 16 recordings (N=8 canines) were performed with bipolar cuff electrodes on the hypogastric nerve during bladder filling with saline at 60ml/min. Compound action potentials were recorded in 10/16 recordings before hypogastric nerve transection. In another 4/16 recordings, the hypogastric nerves were transected between the spinal cord and the electrode, to eliminate efferent nerve signals before recording afferent nerve discharges. The remaining 2 recordings were performed within the abdomen without any contact of cuff on the nerve. We also stimulated the L2 spinal root before and after hypogastric nerve transection (N=3) canines. All recordings were performed using a low noise amplifier (SR560, SR Systems) at 10k gain, 20kHz, filtered (300Hz-10kHz), interfaced with PowerLab (AD Instruments) and LabChart software. Hypogastric nerves were harvested from 5 other canines, cryosectioned and examined for expression of adrenergic marker enzyme tyrosine hydroxylase using immunohistochemistry.

Results: Electrical stimulation of the hypogastric nerve caused an increase in detrusor pressure upto 9 cm H_20. We found that the amplitude of hypogastric nerve activity decreased substantially in response to bladder filling in 6/10 recordings, increased moderately in 1/10 recording, and showed no response in 2/10 recordings. One recording was affected by surrounding radio signal due to a poor ground connection. After nerve transection between the recording electrode and the spinal cord, afferent activity decreased in 3/4 recordings during bladder filling, with no change in 1 recording. No change was observed in the 2 recordings in which the cuff was not in contact with the nerve. L2 spinal root stimulation increased detrusor pressure that was unaffected by bilateral transection of all spinal roots below L5 but was reduced and not completely eliminated by hypogastric nerve transection in all three canines. Each collected hypogastric nerve showed positive tyrosine hydroxylase immunostaining, confirming them as sympathetic.

Conclusions: We observed changes in hypogastric nerve activity during bladder filling that were absent when there was no contact between the nerve and the recording electrode. Based on these results, this technique may be appropriate for recording afferent nerve activity during bladder filling in animals with surgically rerouted neural pathways. L2 root stimulation increases bladder pressure that is not mediated by effects on the lumbosacral spine and only partially mediated by hypogastric nerves.

Funding Source(s): NIH 1R01NS070267

S8A22

Title: Ex vivo penile vascular function is preserved in male C57Bl6/N mice following a 12 week high fat diet despite diet-induced obesity

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Introduction/Objectives: Erectile dysfunction (ED) has been reported in 79% of obese men. Both obesity and ED share endothelial damage as a common etiology that reduces nitric oxide (NO) availability and results in impaired vasodilation. The majority of high fat diet (HFD) mouse models use the C57Bl/6J strain which develop endothelial dysfunction after 12 weeks of diet. Although commonly used, the current C57Bl/6J strain has been shown to possess over 200 genetic mutations from the original C57Bl/6J mouse. A novel C57Bl/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 C57Bl/6N mice.

Methods: 10 week old male C57Bl/6N mice were fed a 45% HFD or 10% fat control (CON) diet for 6 or 12 weeks (n=10 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 6 and 12 weeks. Aortas, internal pudendal arteries (IPA), and penises were excised and mounted in myographs (n=6 per group). Smooth muscle contraction was assessed via high dose 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 of DEA NONOate (NO donor) and 8-pcpt-cGMP (cGMP analogue). Endothelial dependent relaxation to acetylcholine (Ach; muscarinic agonist) was also evaluated.

Results: Following 6 weeks of HFD, mice had increased body weight by 25%, increased body fat by 81%, and elevated blood glucose by 45% following GTT compared to age matched CON mice. Both endothelial dependent and independent relaxation was unchanged after 6 weeks HFD in the aorta, IPA and penis. KCl contraction was significantly decreased ($p<0.001$) in 6 week HFD aortas; however, PE and ET-1 CRCs were unchanged in all tissues. After 12 weeks of HFD, mice had further increased body weight by 37%, increased body fat by 111%, and elevated blood glucose by 42% following GTT compared to age matched CON mice. Surprisingly after 12 weeks of HFD, endothelial dependent and independent relaxations were still unchanged from age matched CON. Contractile responses to KCl and PE were significantly decreased in the aortas from 12 week HFD mice. In contrast, no changes in KCl, PE or ET-1 induced contraction was evident in penises or IPA from 12 week HFD mice.

Conclusions: Male C57Bl/6N mice fed a HFD develop marked increased adiposity and impaired glucose metabolism yet are protected from penile vascular damage. Only the aorta after 12 weeks of HFD began to show signs of impaired contractility. Further evaluation of the genetic and phenotypic differences between C57Bl/6N and C57Bl/6J is warranted to identify underlying vascular protective mechanisms which may yield new therapeutic targets in the treatment of obesity induced vascular dysfunction in humans.

Funding Source(s): Startup funds BSOM.

S8A23

Title: Evidence of hyperglycemic memory following diabetic bladder disease.

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Introduction/Objectives: Diabetic Bladder Disease (DBD) encompasses several pathologies which include urge and flow incontinence. Current treatments are limited to glycemic control and medications that treat clinical symptoms. However, after prolonged hyperglycemia DBD is difficult to reverse, even if normal glucose levels are achieved. In other pathologies associated with diabetes, the inability of glycemic control to reverse pathology is well documented (such as diabetic retinopathy and neuropathy and macrovascular complications) and known as "hyperglycemic memory". Hyperglycemic-memory is associated with changes in metabolism that modify genomic DNA (epigenomic modification). The objectives of the studies presented here were to demonstrate that prolonged hyperglycemia changes bladder metabolism resulting in irreversible modification of genomic DNA.

Methods: Experimental protocols were approved by our Institutional Animal Care and Use Committee. Five-male F344 rats were made diabetic for 4-months using streptozotocin treatment (STZ- a model of Type 1 diabetes). A second group of 5-male F344 rats were made diabetic for 3-months followed by 1-month of intensive insulin treatment. A control group consisted of 5-non-diabetic, age matched controls. At the end of the study animals were euthanized and bladder mucosal and detrusor tissue isolated for metabolomic and epigenomic profiling. ANOVA was used to identify significant changes in the expression of metabolites and methylation pattern of specific genetic loci.

Results: Diabetes results in significant changes in metabolism, several of these changes could result in epigenomic modification (such as increased pathways leading to oxidative stress, and decreased methionine and betaine levels causing a "methyl-donor substrate' deficiency which could lead to decreased methylation of genomic DNA). Most (but not all) changes in metabolism are reversed by insulin treatment. Epigenomics demonstrated those metabolic pathways that are not reversed by insulin treatment predominantly contain genes that undergo epigenomic modification due to hyperglycemia that are also not reversed by insulin treatment. Some of these genes play important roles in bladder physiology such as specific lipid membrane components and potassium channels.

Conclusions: We provide evidence of "hyperglycemic memory" in the bladder: Prolonged hyperglycemia changes bladder metabolism resulting in irreversible modification of genomic DNA. The "genes" responsible for hyperglycemic memory would represent the best targets for treatment of patients that have diabetic bladder disease recalcitrant to glycemic control**.**

Funding Source(s): Lilly Innovation Fellowship Award, NIH/NIDDK (DK-107807) and DiaComp Grants.

S8A24

Title: Regulation of tone in the IAS by phasic events: role of L-type calcium channels and stretch.

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Introduction/Objectives: The IAS is a phasic muscle that generates tone. We have hypothesized that slow waves (SWs) generated by interstitial cells of Cajal (ICC) conduct to adjacent smooth muscle cells (SMCs) where L-type calcium channels (Cav_L) are activated resulting in phasic contractile activity that sums to generate tone. The present study addressed this hypothesis by examining the origin and spread of calcium transients in the IAS and the relationship of this activity to Cav_L.

Methods: The spatiotemporal pattern of calcium transients was visualized in SMCs of the SM-GCaMP3 mouse IAS with confocal microscopy. Inhibitory motor neurons were stimulated with electrical field stimulation (EFS) in the presence of atropine (1 μM) and guanethidine (1 μM). SWs were recorded at various distances away from the distal edge of the wildtype mouse IAS while summed contractile activity was recorded in isolated muscle strips.

Results: Confocal imaging at low magnification (4x) revealed calcium transients that predominantly arise from the distal extremity of the IAS and conduct proximally for distances ≥300 μm. The average frequency of calcium transients at the distal edge of the IAS was 79 cycles per minute while the time constant for the decline of these transients averaged 0.34 seconds. The level of calcium between phasic contractions was determined by abolishing contraction with EFS. Basal calcium associated with tone development was found to be significantly above calcium levels in the absence of contractile activity. Calcium transients and SWs persisted following removal of the distal most 0.3 mm of the IAS, however, the frequency of these events was lower than in intact muscles. Tone and phasic contractions also persisted following removal of the distal most 0.3 mm. These data suggest that pacemaker cells are not confined to the distal extremity of the IAS but rather are distributed throughout the muscle. SWs, contraction and calcium transients were all abolished by the Cav_L blocker nifedipine (0.1 μM) and by hyperpolarizing membrane potential with the K_{ATP} channel activator pinacidil (10 μM). Stretch was not required for generation of SWs, tone or calcium transients but a small increase in the conduction velocity of calcium transients was observed in stretched muscles.

Conclusions: These data support the hypothesis that tone results from the summation of phasic events and that calcium entry via Cav_L is essential for the generation of tone while stretch is not. The predominant pacemaker is at the distal extremity but additional slower pacemakers are also present, much like the heart where the SA node is the predominant pacemaker. In the case of the IAS, this phasic activity serves to generate tone and maintain fecal continence.

Funding Source(s): NIH DK078736

PS25

Title: Bladder Expression of Myosin 5a Isoforms

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Introduction/Objectives: Myosin 5a (Myo5a) is a motor protein required for short-range transport of molecular cargo. In neurons, it transports neurotransmitter-containing synaptic vesicles to the varicosity membrane, while in skin melanocytes it transports pigment-containing vesicles. DBA mice have a defect in Myo5a expression, with the dilute coat color but without the fatal neurologic abnormalities seen in mice having complete deletion of the Myo5a gene. Their survival is thought to result from normal expression of the Myo5a "brain" isoform in neurons. However, we have shown that bladder tissue (BSM) of DBA mice exhibits an impaired response to electrical field stimulation, compared to that of wild-type C57 mice. This impairment is potentially due to reduced release of neurotransmitter-containing vesicles, rather than to reduced responsiveness to neurotransmitters. The aim of the current study was to investigate Myo5a isoform expression in DBA and C57 bladders to better understand differential responsiveness to EFS.

Methods: Total RNA was extracted from DBA and C57 bladder muscle without mucosa, converted into cDNA, and used in nested PCR to amplify the alternative exon region of Myo5a mRNA, which defines the isoforms expressed. PCR product size and restriction enzyme digestion patterns were determined by agarose gel electrophoresis. Myo5a protein was extracted from C57 BSM, separated by denaturing polyacrylamide electrophoresis, and Western blotted with anti-Myo5a antibodies.

Results: PCR and restriction analysis revealed that C57 BSM expresses at least two Myo5a isoforms, one of which is the brain isoform with alternative exon sequence ABCE, while the other is a skin isoform of exon sequence ACDEF, ACDE and/or ACEF. In contrast, DBA BSM expressed predominantly ABCE but very little of the other isoforms. Protein analysis confirmed expression of multiple Myo5a isoforms in C57 BSM. Four Myo5a bands differing in electrophoretic mobility were detected by Western blot. The fastest-mobility band comigrated with brain Myo5a, while two additional bands comigrated with Myo5a isoforms from skin.

Conclusions: Several Myo5a isoforms are expressed in bladder at the mRNA and protein levels. The neurogenic change seen in DBA contractility to electrical field stimulation suggest a functional role for multiple Myo5a isoforms in bladder neurotransmission, including a nerve isoform that is distinct from the brain form.

Funding Source(s): Department of Veteran Affairs, Research Service, Washington DC.

PS26

Title: Ca^{2+}-dependent activation of transient receptor potential melastatin-4 channels by sarcoplasmic reticulum inositol trisphosphate receptors in human detrusor

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Introduction/Objectives: Recently, our group reported that the Ca^{2+}-activated transient receptor potential melastatin-4 (TRPM4) channels play a key physiological role in maintaining the resting membrane potential in detrusor smooth muscle (DSM). However, the Ca^{2+}-signaling mechanisms governing TRPM4 channel activity in human DSM cells remained unexplored. As the TRPM4 channels are activated by Ca^{2+}, inositol 1,4,5-trisphosphate receptor (IP_3R)-mediated Ca^{2+} release from the sarcoplasmic reticulum (SR) represents a potential Ca^{2+} source for TRPM4 channel activation.

Methods: To investigate the molecular and functional interactions of the SR IP_3Rs and TRPM4 channels, clinically-characterized human DSM tissues were acquired from donor patients undergoing routine open bladder surgeries. With *in situ* proximity ligation assay (PLA) and perforated patch-clamp electrophysiology, we tested the hypothesis that TRPM4 channels are tightly associated with the IP_3Rs and are activated by IP_3R-mediated Ca^{2+} release in human DSM.

Results: Using *in situ* PLA, we demonstrated co-localization of the TRPM4 channels and SR IP_3Rs in freshly-isolated human DSM cells. As the TRPM4 channels and SR IP_3Rs must be located within close apposition to functionally interact, these findings support the concept of a potential Ca^{2+}-mediated TRPM4-IP_3R regulatory mechanism. To investigate SR IP_3R regulation of TRPM4 channel activity, we sought to determine the consequences of IP_3R pharmacological inhibition on TRPM4 channel-mediated transient inward cation currents (TICCs). In freshly-isolated human DSM cells, blocking the IP_3Rs with the selective IP_3R inhibitor xestospongin-C (1 μM) significantly decreased TICC activity (NP_o) at the holding potential of -70 mV. Under control conditions TRPM4 NP_o was 2.0±1.1, which was reduced to 1.3±1.0 in the presence of 1 μM xestospongin-C (n=6, N=4; $P<0.05$). These results support the concept that Ca^{2+} release events from SR IP_3Rs are involved in the regulation of TRPM4 channel-mediated TICCs in human DSM cells.

Conclusions: This study provides novel mechanistic insight into the molecular and cellular mechanisms regulating TRPM4 channels by revealing that TRPM4 channels and SR IP_3Rs are spatially and functionally coupled in human DSM. The data further suggest that IP_3Rs have a key role in mediating the Ca^{2+}-dependent activation of TRPM4 channels in human DSM.

Funding Sources: NIH F31 DK104528, NIH R01 DK106964.

PS27

Title: Direct innervation from lower thoracic and upper lumbar ventral horn to the detrusor is mediated by neuromuscular nicotinic receptors

Authors: Danielle M. Salvadeo[1], Ekta Tiwari[2], Nagat Frara[1], Alan S. Braverman[1], Mary F. Barbe[1], Michael R. Ruggieri, Sr. [1,2,3]

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Introduction/Objectives: It is well established that the bladder receives parasympathetic innervation from the sacral spinal cord, as well sympathetic innervation from the lower thoracic and upper lumbar spinal cord. Additionally, we previously conducted a retrograde tracing study in which we found that the detrusor is innervated by a small number of direct inputs originating in the lower thoracic and upper lumbar ventral horns. We sought to further characterize these direct fibers through *in vivo* and *ex vivo* pharmacological experiments.

Methods: Female mixed-breed hounds were assigned to either a sham-operated/control group (n=7) or a sacral root transection group (n=6), which included bilateral transection of all roots caudal to spinal level L7, as well as the hypogastric nerves. At terminal surgery 8-12 months following the initial procedure, animals received a laminectomy from spinal level T10 to S4. Roots originating from each level were stimulated and changes in pressure were recorded. Three of seven control animals underwent additional testing whereby the neuromuscular nicotinic receptor antagonist atracurium was administered and lower thoracic/upper lumbar roots were stimulated. Data was analyzed using an unpaired t-test.

At euthanasia, the bladder was harvested and strips of smooth muscle devoid of mucosa were isolated from bladders. The contractile response to electric field stimulation was determined in the presence of 5 µM atracurium (n=18-24 strips from 5 animals). Data was analyzed using 2-way ANOVA followed by Tukey's multiple comparisons test.

Results: Animals from the sham-operated group and the transection group exhibited detrusor contractions from lower thoracic/upper lumbar stimulation. IV administration of 25 mg/kg atracurium significantly blocked detrusor contraction induced by stimulation of L2 ventral root *in* vivo ($p=0.0029$; n=3) In contrast, treatment with 5 µM atracurium did not block EFS-induced contractions in isolated detrusor smooth muscle strips *ex vivo*.

Conclusions: The presence of detrusor contractions after lower thoracic/upper lumbar root stimulation regardless of sacral root and hypogastric nerve transection suggests that the contractions are mediated by nerves other than the traditional sacral parasympathetic or hypogastric sympathetic innervation. A significant decrease in detrusor contraction after treatment with atracurium indicates that activity of this subpopulation of nerves is mediated by neuromuscular nicotinic receptors. Furthermore, the nicotinic receptors are not located in the intramural ganglia due to absence of change in contractility of isolated bladder smooth muscle after treatment with atracurium *ex vivo*. Functional motor innervation of the bladder from above the lumbosacral spinal cord may provide new options for treating urinary incontinence following lower spinal cord injury.

Funding Source(s): NIH-NINDS NS070267

PS28

Abstract was Moved to Oral Presentation

PS29

Title: Inflammatory cytokine interleukin 1 beta increases apoptosis in dissociated major pelvic ganglia neurons

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Introduction/Objectives: Bladder outlet obstruction (BOO) is a widespread condition resulting from benign prostatic hyperplasia in men. Long term BOO can result in bladder denervation and ensuing bladder complications such as incontinence, urinary tract infections, and renal failure. Recent research suggests that inflammation is one of the major aggressors leading to denervation of the bladder smooth muscle and resulting incontinence. Interleukin 1 beta (IL-1β) is a cytokine that is increased in the rat model of BOO and inhibition of IL-1β receptors in BOO models prevented bladder denervation. Our study examined the effects of IL-1β on growth and survival of neurons from the major pelvic ganglia (MPG) which innervate the bladder. We hypothesized that IL-1β would lead to increased neuronal apoptosis and decreased axon length and number of branches.

Methods: MPGs were carefully dissected from healthy adult Sprague Dawley male rats (n=4). MPG neurons were dissociated and plated on laminin coated coverslips. Neurons were attached to coverslips for 24 hours and then exposed to concentrations of IL-1β (0, 100, 250, 500 ng/ml). 48 hours later, neurons were fixed and stained with immunofluorescence for neuron-specific class III beta-tubulin to identify neurons and measure axon length and branching. Neurons were co-stained with TUNEL assay to identify apoptotic cells. Images were taken at 100x magnification for analysis. Apoptotic cells were counted and normalized to the total number of neurons and axon lengths and number of branches were measured. Data are reported as the mean ± standard error (SEM). Statistical analyses performed were a one-way ANOVA with a Tukey's post-hoc test using GraphPad Prism software. Results were considered statistically significant at $P < 0.05$.

Results: Increasing concentrations of IL-1β led to a concentration dependent increase in neuronal apoptosis (0ng: 6.35%, 100ng: 20.95%, 250ng: 30.82%, 500ng: 45.00% apoptotic/total cells; $p<0.05$). The average neurite length also increased concentration dependently with IL-1β (100ng: 12.31%, 250ng: 18.49%, 500ng: 31.57% relative to controls). In contrast, the number of branches did not change with increasing IL-1β concentrations.

Conclusions: This data supports the hypothesis that excess IL-1β leads to denervation of bladder smooth muscle in BOO. IL-1β caused a concentration dependent increase in apoptosis and neurite length in neurons from the MPG. Preventing the increased apoptosis via inhibition of IL-1β may be an effective therapy to prevent neuronal loss in BOO leading to incontinence and merits further investigation.

Funding Source(s): R01DK103534, and internal Duke University and East Carolina University funds.

PS30

Title: Lumbar to sacral root rerouting to restore bladder function in a feline spinal cord injury model: urodynamic and retrograde nerve tracing results from a pilot study

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Introduction/Objectives: Lumbar to sacral rerouting surgery can potentially allow voiding via a skin-central nervous system-bladder reflex pathway. Here, we assessed if this surgery was effective in treating neurogenic bladder/sphincter dysfunction in felines.

Methods: Eight cats underwent spinal cord transection (SCT) at thoracic level 10/11. Unilateral L7 to S1 ventral root anastomosis was performed 1 month later in 6 cats. Two cats served as transection-only controls. Electrical and manual stimulation of L6-S1 dermatomes, and urodynamics were performed at 3, 5, 7 and 9/10 months post transection. At 9/10 months, cats were also evaluated by direct electrophysiological testing of anastomosed roots with urodynamics, then tissue collection and examination of the root anastomosis site and lumbosacral cord ventral horns for cells retrogradely labeled from tracer dye injected two weeks earlier into the bladder wall.

Results: At 9/10 months, 4 of 6 rerouted cats exhibited increased detrusor pressure provoked by cutaneous stimulation, one cat bilaterally. Two cats presented with a voiding stream after ipsilateral cutaneous stimulation at 7 and 9 months. All 6 rerouted animals showed regrowth of axons from the L7 ventral horn to the bladder, although some aberrant axonal regrowth was also observed.

Conclusions: L7 to S1 ventral root rerouting below the level of SCT showed successful axonal regrowth to the bladder from the L7 spinal cord segment in all rerouted animals, and induced increased detrusor pressure response to cutaneous stimulation in a subset. This feasibility study paves the way for future animal studies for bladder reinnervation.

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PS31

Title: Peripheral nerve neuromodulation of urological function using a distributed sensing and stimulation system

Authors: Lance Zirpel, Jeff Herron, Thaddeus Brink, Ben Isaacson, Scott Stanslaski, Tom Adamski, Tim Denison

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Introduction/Objectives: Data from previous studies have demonstrated that stimulation of the lumbar/sacral nerves in rodents and sacral nerves in sheep during the latter half of the bladder filling cycle elicited an increase in bladder capacity equal to that elicited by continuous stimulation. These results suggest that nerve stimulation temporally linked to the latter 50% of the bladder fill cycle can provide more efficient urological effects with respect to energy consumption and a reduction in unnecessary stimulation of the nerves. The goal of this study was to demonstrate that signals from a bladder voiding event could be used to control a neurostimulator delivering sacral nerve stimulation in fully conscious, unrestrained ovine.

Methods: Four adult, female, Polypay sheep were implanted with DSI L21 large animal telemetry-based sensors (Data Sciences International, St Paul MN, USA) to continuously monitor bladder pressure (BP), intra-abdominal pressure, detrusor electromyograph, body temperature, and activity (accelerometry) in real time. Animals were also implanted with Medtronic research neurostimulators (Activa RC+S) connected to bilateral sacral leads adjacent to S3 or S4 nerves.

Results: Using the commercial data acquisition software (Ponemah®, Data Sciences International, St. Paul, MN, US), BP and intra-abdominal pressure were used to identify voids with 80% accuracy (validated via video). The voiding event triggered a signal to be sent to a custom research application programming interface (Summit Research API, Medtronic) running on a laptop computer controlling the Activa RC+S neurostimulator via Bluetooth. The neurostimulator responded to the signal by terminating stimulation for a programmed duration after which stimulation was resumed. Activa RC+S status was monitored in real time and stimulation on/off was verified by presence or absence of visually identified motor responses.

Conclusions: The results of this study demonstrate the ability of a distributed system to use physiological signals sensed from the end organ to control neurostimulation of target nerves that are anatomically distinct and distant from the end organ impacted by the stimulation. Specifically, BP signals obtained from a sensor in the bladder were wirelessly transmitted to a computer that determined “void” or “not void”. When a void was identified, another signal was sent to a second computer running the Summit Research API which wirelessly communicated an “off” command to the Activa RC+S. The neurostimulator was programmed respond to this “off” command by terminating stimulation of the sacral nerves for a predefined time interval. By tailoring this interval to either average or individual inter-contractile intervals, SNS can be temporally targeted to physiologically-relevant phases of the bladder fill cycle based on a local physiomarker. This system provides a framework in which any sensor that monitors a quantifiable biomarker can provide physiologically relevant input to a neuromodulation system to specifically adjust stimulation in a manner that is outcome-dependent.

Funding Source(s): All authors are employees of, and the study was funded by, Medtronic.

PS32

Title: Comparison of bladder shape between individuals with and without overactive bladder

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Affiliations: [1]Department of Mechanical & Nuclear Engineering, [2]Department of Urogynecology, [3]Department of Biomedical Engineering, [4]Department of Surgery, and [5]Department of Radiology. All: Virginia Commonwealth University, Richmond, VA.

Introduction/Objectives: Overactive bladder (OAB) is characterized by changes in the filling phase of the bladder. Changes in geometry during filling may play a role in the development of bladder urgency, because tension sensitive afferent nerves relay filling sensation. The purpose of this study was to describe dynamic changes in bladder shape over filling, and to compare the pattern of filling between OAB patients and volunteers with normal bladder function.

Methods: Fourteen female patients with OAB and twelve healthy volunteers (7 male, 5 female) were enrolled in this prospective study. The OAB group underwent extended urodynamic testing with transabdominal 3D ultrasound. Images were acquired once every minute during bladder filling at an infusion rate of 10% cystometric capacity per minute. The normal group drank 2L Gatorade-G2 and had images acquired every five minutes as their bladders filled by ureteric diuresis. Perimeters were measured manually in the transverse, sagittal, and coronal planes.

Results: **Figure 1** shows how average bladder perimeter increased in the three planes for the normal (left) and OAB (right) groups. In the normal group, the largest change was seen in the transverse plane, which became much larger than the perimeters of the other planes over the course of filling. In contrast, in the OAB group, the largest degree of change was seen in the sagittal plane. By the end of filling, the perimeters in all three planes were nearly identical.

Conclusions: At the end of filling in the OABs, the perimeters in all planes were approximately equal, implying that the bladder had become more uniform over the course of filling, a pattern not seen in the normal group. Final perimeters in the normal group were larger than in the OAB group, because this group had larger bladder capacities. Altered bladder filling geometry changes might identify some patients likely to develop OAB.

Funding Source(s): Support provided by NIH R01DK101719

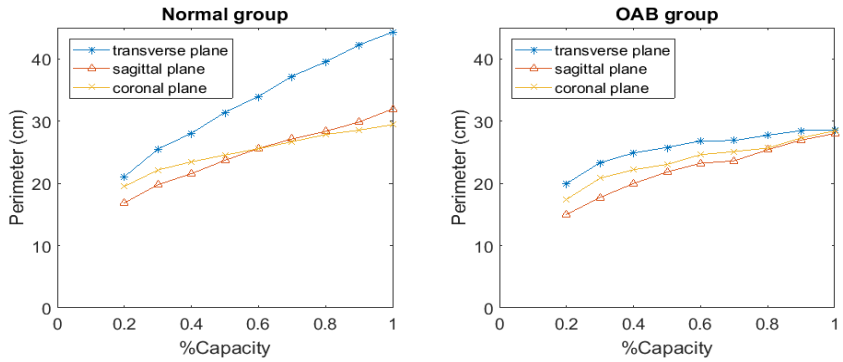

Figure 1. Average perimeter in the transverse (blue stars), sagittal (orange triangles), and coronal (yellow crosses) for the normal (left) and OAB (right) groups as a function of %capacity (normalized volume).

PS33

Title: Investigation of Motor Unit Firing Patterns of Bulbospongiosus Muscle in Type II Diabetic Female Patients

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Introduction/Objectives: Diabetic neuropathy is a common complication impacting central, peripheral and autonomic nervous system in diabetes mellitus (DM) patients. Limited efforts were made to investigate the functional properties of motor units (MUs) of pelvic muscles in diabetic neuropathy patients. This is possibly because of the complex pelvic anatomy make it difficult to access the muscles and their innervating nerve. In this study, we use an intra-vaginal high-density electromyography (EMG) probe to study the effect of diabetic neuropathy on the firing patterns of bulbospongiosus muscle in female.

Methods: Surface EMG was recorded during voluntary pelvic contraction using a 64-channel (8*8) high-density intra-vaginal probe, from both aged healthy (n=6, 60±4 years) and type 2 non-insulin dependent DM group (n=4, 62±2 years). Surface interferential HD-EMG recordings were then decomposed to constitutive motor unit action potential trains, using our developed K-means clustering convolution kernel compensation (KmCKC) algorithm. Firing rate (FR) was quantified as the average instance of MU discharging per second for all decomposed MUs. The mean FR was considered to represent averaged discharge rate of the entire MU pool in bulbospongiosus muscle.

Results: EMG decomposition was successfully performed in all 10 subjects. A mean FR of 13.8±1.7 and 10.8±2.1 was calculated for healthy and DM group, respectively. A significant difference in FR was found between two groups using a two-tailed student's t-test ($p<0.05$).

Conclusions: Although the small subject pool studied may not yield desired statistical power, preliminary results have shown a marked FR decrease in DM patients, suggesting compromised capacity of MU recruitment. It can be caused by the compromised excitatory drives from motor cortex to the lower motor neurons or alterations in the peripheral nervous excitability. This work also exhibit, to the best of our knowledge, the first effort in minimum invasively investigating firing patterns of superficial pelvic muscles in DM patients, by overcoming the complex pelvic anatomy using our high-density EMG intra-vaginal probe. Similar neuromuscular disorders can potentially as well affect other neighboring pelvic musculatures, such as urethra sphincter and anal sphincter, consequently impairing the urinary and fecal continence. The results may also broaden the potential applicability of presented approach for clinical investigation of neuromuscular dysfunctions in pelvic musculatures.

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PS34

Title: Sonic hedgehog regulation of rhabdosphincter muscle.

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Introduction/Objectives: Removal and injury of rhabdosphincter muscle during prostatectomy surgery is a leading cause of stress urinary incontinence (SUI, 44%), which critically impacts patient mental and physical health. With current treatments, including implantation of artificial urinary sphincter, continence pad use is needed and device failure, erosion of the urethra and infection are significant side effects. Thus a critical unmet need exists to develop novel methods to regenerate rhabdosphincter muscle. We have identified sonic hedgehog (SHH) as an important regulator of muscle in another urogenital organ, the penis, and have developed innovative peptide amphiphile nanofiber hydrogel delivery of SHH protein to regenerate penile smooth muscle, post prostatectomy. If similar SHH signaling mechanisms regulate rhabdosphincter function, then this technology may be applied to regenerate rhabdosphincter muscle post prostatectomy. We hypothesize that SHH protein is critical for human rhabdosphincter homeostasis and regeneration and have examined this hypothesis in human rhabdosphincter tissue.

Methods: Human rhabdosphincter (n=5) was obtained from patients (n=5) undergoing cystectomy. Trichrome stain, immunohistochemical analysis for SHH pathway, and cell culture of human rhabdosphincter muscle with SHH protein and inhibitors treatment, was performed.

Results: Trichrome stain showed that human rhabdosphincter is a complex mixture of collagen, muscle and elastin fibers. SHH protein was abundantly expressed in rhabdosphincter muscle. The SHH receptor Patched (PTCH1) was also identified, and strongly stained rhabdosphincter muscle. Rhabdosphincter muscle grew more abundantly in the presence of SHH protein, and reduced growth was observed with two SHH inhibitors.

Conclusions: The SHH pathway is active in adult human rhabdosphincter muscle, and influences muscle growth and homeostasis, suggesting that the SHH pathway may be useful to promote rhabdosphincter regeneration and to treat/prevent SUI.

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PS35

Title: The Ca^{2+}-activated Cl^- channel ANO1 is expressed in mouse urethral interstitial cells of Cajal and contributes to urethral smooth muscle Ca^{2+} signaling and contractility

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Introduction/Objectives: Urethral smooth muscle (USM) generates myogenic contractions to maintain continence during bladder filling. The smooth muscle walls of the urethra are made up of heterogeneous cell populations containing urethral smooth muscle cells (USMC) as well as Kit^+ interstitial cells of Cajal (ICC). Previous studies have suggested that activation of Cl^- current via the Ca^{2+}-activated Cl^- channel ANO1 can affect USM contractions, although there is debate on whether ANO1 is expressed in USMC or ICC. Using cell specific fluorescent reporter animals, we sought to clarify the cellular identity of ANO1 expressing cells in the mouse urethra and to examine its contribution to USM contractility.

Methods: Genes of interest were collected from specific cell populations by the use of cell specific fluorescent reporter mice. Urethral cells were enzymatically isolated from each tissue (SMHC-Cre-eGFP for USMC, Kit-Cre-Tdt-Tomato for ICC) and collected via fluorescence activated cell sorting for qPCR analysis. Isometric tension experiments were carried out on urethral ring preparations from C57 wild-type mice. *In situ* Ca^{2+} imaging was performed using genetically encoded Ca^{2+} indicators driven by cell specific promoters in USMC (SmHC-Cre-GCaMP3) and ICC (Kit-Cre-GCaMP6F) via the Cre-Lox P system.

Results: qPCR analysis revealed that USMC readily express smooth muscle myosin (SMMY) with negligible expression of Kit and PDGFRα. In contrast, sorted cell populations from the Kit-Cre-Tdt-Tomato mice showed minimal expression of SMMY but enriched expression of Kit. Sorted cells from the Kit-Cre-Tdt-Tomato (ICC) mouse did show a 2-fold increase in PDGFRα expression compared to unsorted cells. Only ICC showed abundant enriched expression of ANO1, while there was no difference in expression between sorted USMC and unsorted urethral cells. The selective ANO1 blocker CaCCinh-A01 (10 – 30 μM) significantly reduced USM contractions induced by phenylephrine (PE). CaCCinh-A01 (10μM) also reduced spontaneous Ca^{2+} waves recorded *in situ* in SmHC-Cre-GCaMP3 mice but not in Kit-Cre-GCaMP6F mice.

Conclusions: The mouse urethra contains a heterogeneous cellular population consisting of both USMC and ICC. ICC impart excitable input to USMC via activation of ANO1 and thus contribute to the generation of USM contractions and USMC Ca^{2+} signals.

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