THE SOCIETY FOR PELVIC RESEARCH

FIRST ANNUAL MEETING

MEETING PROGRAM

December 5-6, 2016
Charleston, SC
Sponsored by:
Generous donations by the Glickman Urological & Kidney Institute at Cleveland Clinic

and Medtronic

Special Thanks to:

Ms. Elizabeth Foss CTC, MCC, MBA, President of My Travel Elf, Inc. / MTE Vacations, Naples, FL. 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 generously donated her time and expertise.

Susan C. Fecho MFA, Professor and Dean of the School of Visual, Performing, and Communication Arts at Barton College in Wilson, NC. Susan put the finishing touches on the updated SPR logo. She generously donated her time and expertise.

Monica Liebert PhD, Adjunct Professor, University of Michigan, who advised us early on in the formation of the society, and was instrumental in helping us get launched. She generously donated her time and expertise.

Andrew Foster JD, the Director of the Community Enterprise Clinic at Duke Law School, oversaw then law student Michelle Zheng JD (now at Linklaters) who took us through the legal aspects of incorporating the SPR as a non-profit. They both generously donated their time and expertise.

The 2016 SPR Abstract Review/Final Meeting Planning Committee
Matthew O. Fraser, PhD
Margot S. Damaser, PhD
Maryrose P. Sullivan, PhD
Michael R. Ruggieri, Sr., PhD
Kelvin P. Davies, PhD

All those attending and participating in the Inaugural 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 or 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 3 years. 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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Professor, Department of Physiology and Cell Biology, University of Nevada School of Medicine

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Associate Professor, Department of Biomedical Sciences, Cooper Medical School of Rowan University

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**Members at Large**
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Professor
Departments of Medicine and Pharmacology & Chemical Biology
University of Pittsburgh School of Medicine

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

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

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

December 5, 2016

7:30 AM  Continental Breakfast
8:30 AM  Welcome, Opening Remarks, Mission of the SPR - Matthew O. Fraser, PhD
8:35 AM  Session 1: Physiology & Pharmacology - Matthew O. Fraser, PhD Moderator
  Key Note Lecture - Dr. William C. de Groat, PhD
  Q&A
  Oral Presentations - Abstracts S1A1-S1A6
  Q&A

10:40 AM  Break
10:55 AM  Session 2: Muscle & Organ Function - Steven D. Abramowitz, PhD Moderator
  Oral Presentations - Abstracts S2A8-S2A14
  Q&A

12:30 PM  Lunch
1:30 PM  Session 3: Models & Methods in Pelvic Research
  State of the Art Lecture - Dr. Kenton M. Sanders, PhD - Sean M. Ward, PhD Moderator
  Q&A
  Oral Presentations - Abstracts S3A15-S3A21 - Michael R. Ruggieri, Sr., PhD Moderator
  Q&A

3:55 PM  Break
4:10 PM  Session 4: NIH Funding and Career Paths - Margot S. Damaser, PhD Moderator
  Special Guest Lecture - Dr. Tamara G. Bavendam, MD, MS
  Special Guest Lecture - Dr. Grannum R. Sant, MD, FRCS, FACS
  Q&A

5:20 PM  Break
5:30 PM  Poster Session - Wine and Cheese Reception - Michael E. DiSanto, PhD and Steven D. Abramowitz, PhD - Moderators
  Poster Presentations - Abstracts PS36-PS48

7:30 PM  Adjourn for the Day

December 6, 2016

7:30 AM  Continental Breakfast
8:30 AM  Welcome to Day 2 - Margot S. Damaser, PhD
8:35 AM  Session 5: Neuroscience of Pelvic Organs/Floor
  State of the Art Lecture - Dr. Arthur L. Burnett, II, M D, MBA - Carol A. Podlasek, PhD - Moderator
  Q&A
  Oral Presentations - Abstracts S5A22-S5A28 - Kelvin P. Davies, PhD Moderator
  Q&A

10:40 AM  Break
10:55 AM  Session 6: Novel Therapies/Diagnostics for Pelvic Disorders - Maryrose P. Sullivan, PhD - Moderator
  Oral Presentations - Abstracts S6A29-S6A35
  Q&A

12:30 PM  Closing Remarks/Awards - Matthew O. Fraser, PhD, Michael E. DiSanto, PhD and Steven D. Abramowitz, PhD
1:00 PM  Meeting Adjourns
Program in Detail

December 5, 2016

7:30 AM  Continental Breakfast
8:30 AM  0:05  Welcome, Opening Remarks, Mission of the SPR - Matthew O. Fraser, PhD

Session 1: Physiology & Pharmacology - Matthew O. Fraser, PhD - Moderator

8:35 AM  0:40  Keynote Address - Neural circuitry in the lumbar spinal cord contributing to bladder-sphincter coordination - William C. de Groat, PhD

9:15 AM  0:10  Q & A

9:25 AM  0:10  S1A1  Mechanosensitive release of ATP and other purines from the bladder urothelium - Violeta N. Mutafova-Yambolieva, MD, PhD

9:35 AM  0:10  S1A2  Influence of Mucosal Remodeling on Urothelial Functions - Lori A. Birder, PhD

9:45 AM  0:10  S1A3  In vitro evaluation of hydrostatic pressure on ATP release and Caspase-1 activation in rat urothelial cells - Cody L. Dunton, PhD Student

9:55 AM  0:10  S1A4  Myosalpinx Contractions Are Essential For Egg/Embryo Transport In The Oviduct - Sean M. Ward, PhD

10:05 AM  0:10  S1A5  Purinergic regulation of nitric oxide mediated vasodilation in rat internal pudendal arteries - Michael R. Odom, PhD Student

10:15 AM  0:10  S1A6  Fibrosis in the bladder in response to outlet obstruction is triggered through the NLRP3 inflammasome and the production of IL-1β - Francis M. Hughes, Jr., PhD

10:25 AM  0:15  Q & A

10:40 AM  0:15  Break

Session 2: Muscle & Organ Function - Sang Don Koh, PhD - Moderator

10:55 AM  0:20  S2A8  Selected for Extended Presentation: Altered S-Nitrosation of Contractile Proteins Underlies Dysfunctional Quiescence in Human Preterm Labor - Iain L. O. Buxton, PhD, PharmD

11:15 AM  0:10  S2A9  A method to quantify of bladder wall biomechanics using ultrasound imaging in conjunction with urodynamics - Anna S. Nagle, PhD

11:25 AM  0:10  S2A10  Down-regulation of ryanodine receptor gene expression in murine urinary bladder smooth muscle following partial bladder outlet obstruction - Ettickan Boopathi, PhD

11:35 AM  0:10  S2A11  What’s Wrong with UAB Patients? - Phillip P. Smith, MD
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**Session 3: Models & Methods in Pelvic Research** - Sean M. Ward, PhD and Michael R. Ruggieri, Sr., PhD - Moderators

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<td>1:30 PM</td>
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<td>State of the Art Lecture - Use of Optogenetic Sensors and Actuators to Study the Roles of Specific Cells in Intact Visceral Smooth Muscles - Kenton M. Sanders, PhD</td>
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Session 4: NIH Funding and Career Paths – Margot S. Damaser, PhD - Moderator

4:00 PM 0:30 Special Guest Lecture - Opportunities for Funding Cross-Disciplinary Pelvic Research - Tamara G. Bavendam, MD, MS

4:30 PM 0:30 Special Guest Lecture - Transition from Academia to Industry - Pathway and Opportunities - Grannum R. Sant, MD, FRCS, FACS

5:00 PM 0:15 Q & A

5:15 PM 0:15 Break

5:30 PM 2:00 Poster Session / Wine and Cheese Reception - Michael E. DiSanto, PhD and Sang Don Koh, PhD - Moderators

PSA36 Loss of nitric oxide-mediated inhibition of purine neurotransmitter release in the colon in the absence of interstitial cells of Cajal - Leonie Durnin, PhD

PSA37 Cholinergic excitatory motor responses in the colon are mediated through the Calcium-Activated Chloride Conductance Ano1 - Sung Jin Hwang, PhD

PSA38 Clarification of the innervation of genitourinary structures: a neuronal tracing study in female mongrel hound dogs - Mary F. Barbe, PhD

PSA39 The Use of Support Vector Machine in the Prediction of Stress Urinary Incontinence - Brian M. Balog, PhD Student

PSA40 Spontaneous Ca2+ waves in mouse urethral smooth muscle visualized with a genetically encoded Ca2+ indicator in situ - Bernard T. Drumm, PhD

PSA41 Localization of Neuromuscular Nicotinic Receptors in The Functionally Reinnervated Canine Bladder after Prolonged Decentralization - Nagat Frara, PhD

PSA42 Cyclophosphamide-induced Overactive Bladder via Downregulation of Relaxation Factors in Detrusor PDGFRα+ Cells - Haeyeong Lee, PhD

PSA43 Comparison of Bladder Volumes between 2D and 3D Ultrasound Calculations and Urodynamic Measurements in Women with Overactive Bladder (OAB) - Anna S. Nagle, PhD

PSA44 Pharmacological Activation of Individual KCNQ Channel Subtypes in Detrusor Smooth Muscle Represents a Promising Novel Approach for Overactive Bladder Treatment - Aaron Provence, PhD Candidate

PSA45 Decentralization reduces nicotinic receptor-mediated canine bladder contractions in vitro - Danielle M. Salvadeo, MD/PhD Student

PSA46 In-vivo hypogastric nerve electrical stimulation contracts the canine detrusor - Ekta Tiwari, PhD Student

PSA47 Monitoring nerve activity during bladder filling in a rat model - Ekta Tiwari, PhD Student

PSA48 The Effects of Myrbetriq on Detrusor Overactivity associated with Suprasacral Spinal Cord Injury (SCI) in Rats - Matthew O. Fraser, PhD
December 6, 2016

7:30 AM  Continental Breakfast
8:30 AM  0:05  Welcome to Day 2 of the Inaugural SPR Meeting - Margot S. Damaser, PhD

Session 5: Neuroscience of Pelvic Organs/Floor - Carol A. Podlasek, PhD and Kelvin P. Davies, PhD
- Moderators

8:35 AM  0:30  State of the Art Lecture: Nitric Oxide Function and Dysregulation in the Lower Genitourinary Tract: Health and Disease - Dr. Arthur L. Burnett, II, MD, MBA

9:05 AM  0:10  Q & A

9:15 AM  0:10  S5A22  Functional Relevance of Purinergic P2X4R in Bladder Smooth Muscle - Vivian Cristofaro, PhD

9:25 AM  0:10  S5A23  Aberrant bladder reflexes can drive hind limb locomotor activity following complete suprasacral spinal cord injury - Matthew O. Fraser, PhD

9:35 AM  0:10  S5A24  Spinal Cord Injury and Detrusor PDGFRα+ Cells - Haeyeong Lee, PhD

9:45 AM  0:10  S5A25  NLRP3/IL-1β mediates Denervation During Bladder Outlet Obstruction in Rats - Robin Lutof, MS Student

9:55 AM  0:10  S5A26  Micturition and defecation behavior following pelvic decentralization in a canine model - Michael R. Ruggieri, Sr., PhD

10:05 AM  0:10  S5A27  Sonic hedgehog promotes sprouting of neurons in the pelvic ganglia and cavernous nerve during regeneration - Ryan Dobbs, MD

10:25 AM  0:15  Q & A

10:40 AM  0:15  Break

Session 6: Novel Therapies/Diagnostics for Pelvic Disorders - Maryrose P. Sullivan, PhD - Moderator

10:55 AM  0:20  S6A29  Selected for Extended Presentation: Sphingosine-1-phosphate in vitro and in vivo modulates corpus cavernosum smooth muscle tone - Michael E. DiSanto, PhD

11:15 AM  0:10  S6A30  Neurotrophin Dysregulation after Dual Injury Childbirth Model is corrected via Electrical stimulation of the Pudendal Nerve in a Rat Model - Brian M. Balog, PhD Student

11:25 AM  0:10  S6A31  Controlled release of IGF1 enhances urethral sphincter function and histological structure in the treatment of female stress urinary incontinence in a rodent model - Margot S. Damaser, PhD

11:35 AM  0:10  S6A32  Aging Effects on the Central Motor Control of the External Anal Sphincter in Women - Yun Peng, PhD Student
11:45 AM  0:10  S6A33  Systemic Treatment of Stress Urinary Incontinence with Human Urine-Derived Stem Cells - Christine Tran, MD

11:55 AM  0:10  S6A34  Optimization of Sonic hedgehog delivery from self-assembled nanofiber hydrogels - Carol A. Podlasek, PhD

12:05 AM  0:10  S6A35  Sympathetic reinnervation of the urinary bladder using somatic donor nerves in a canine model of lower motoneuron lesioned bladder - Mary F. Barbe, PhD

12:15 AM  0:15  Q & A

12:30 PM  0:30  Closing Remarks/Awards - Matthew O. Fraser, PhD, Michael E. DiSanto, PhD and Sang Don Koh, PhD

1:00 PM  MEETING ADJOURNS
Key Note Speaker

Dr. William C. de Groat, PhD
University of Pittsburgh

William C. de Groat, PhD, is Distinguished Professor in the Department of Pharmacology and Chemical Biology at the University of Pittsburgh School of Medicine. Dr de Groat earned his doctorate in pharmacology from the University of Pennsylvania, Perelman School of Medicine, where he also completed postdoctoral training in pharmacology. He obtained further postdoctoral training in neurophysiology at The John Curtin School of Medical Research in Canberra, Australia.

Dr. de Groat’s research interests include the neural control of the lower urinary tract and the mechanisms underlying urinary incontinence, painful bladder conditions and neurogenic bladder dysfunction after spinal cord injury. He has received a number of awards, including an NIH MERIT Award, an NIH Research Career Development Award, a Lifetime Achievement Award from the Urodynamics Society. Dr de Groat is a Fellow of the American Association for Advancement of Science and has been recognized with the Carl Ludwig Distinguished Lectureship of the American Physiological Society, University of Pittsburgh President’s Distinguished Research Award, Ludwig Guttmann Lectureship at the International Congress of Paraplegia, Reeve-Irvine Prize for Research in the Field of Spinal Cord Injury, Pharmacia-ASPET Award for Experimental Therapeutics from the American Society for Pharmacology and Experimental Therapeutics and the Ferdinand Valentine Award from the New York Academy of Medicine.

Dr. de Groat published over 600 papers in the fields of autonomic neuroscience and neurourology. He has served on numerous editorial boards including The Journal of Pharmacology and Experimental Therapeutics, American Journal of Physiology, Autonomic Neuroscience and Urology. He also has been Treasurer and a member of the Executive Council of the Society for Neuroscience and the Executive Vice President of the International Society for Autonomic Neuroscience. He is an honorary member of both the American and the Japanese Urological Associations.

Neural circuitry in the lumbar spinal cord contributing to bladder-sphincter coordination

External urethral sphincter (EUS) EMG bursting (indicative of rhythmic activity) and coordination between EUS and bladder activity are necessary for efficient voiding in the rat. Spinal cord transections at different levels indicate that this coordination is dependent on a lumbar spinal coordinating center (LSCC) in the L3-L4 lumbar spinal segments, while reflex pathways responsible for tonic activity are located below L4-5. We have studied the organization of the LSCC using pseudorabies virus (PRV) transneuronal tracing and electrophysiological techniques (patch clamp recording in spinal cord slices and spinal cord stimulation in vivo). The results of these experiments provide insights into the organization of the LSCC circuitry and support for the proposed role of the LSCC in generating EUS EMG bursting during voiding.
Dr. Kenton Sanders received his PhD from UCLA and completed post-doctoral work at UCLA and the Mayo Clinic in Rochester, MN. He started his own laboratory in 1979 and began to investigate the electrical mechanisms of spontaneous electrical rhythmicity in visceral smooth muscles. His group isolated and recorded from interstitial cells from GI and bladder muscles, and has developed concepts about what different classes of interstitial cells contribute to organ-level functions in the gastrointestinal and urinary tracts. His lab has also made many contributions regarding the ionic conductances involved in smooth muscle excitability and excitation-contraction coupling, neuromuscular mechanisms and Ca\(^{2+}\) handling mechanisms in smooth muscle cells and interstitial cells. Dr. Sanders has been supported continuously through funding from the NIH since 1977. In addition to numerous R01s and 4 Program grants, he has also received a Research Career Development Award and an NIH MERIT award from the NIDDK. Dr. Sanders is currently Professor and Chair of the Department of Physiology and Cell Biology at the University of Nevada, Reno.

Use of optogenetic sensors and actuators to study the roles of specific cells in intact visceral smooth muscles

Investigations into the physiology of visceral smooth muscle organs began as studies of the integrated behavior of these complex tissues by measuring pressures in the hollow chambers and tubes of the organs and force development of strips of muscle in response to pharmacological substances and putative neurotransmitters. As anatomists described the complexity of these organs, it became necessary for physiologist to devise the means of reducing the tissue into its cellular components to allow closer observations of cell behaviors. From these reductionist studies, great progress was made during the past 30-40 years regarding the mechanisms at play in the various cellular components if these organs.

The nerve pathways and neurotransmitters driving organ-level behaviors are quite well described, and detailed information about cellular responses has been catalogued. But in fact, the behaviors of visceral smooth muscle organs result from the sum of many cells acting via neurotransmission, paracrine mechanisms and electrical coupling, and emergent properties can result from the integration of diverse cellular activities. This level of organization has required a new generation of tissue and organ level investigation, and these pursuits are being aided by many new imaging technologies, including optogenetics. This state-of-the-art talk will describe some of the growing capabilities of optogenetic techniques and provide examples of how these techniques are revealing the behaviors of specific cell-types in intact muscles and visceral smooth muscle organs.
**State of the Art Speaker**

**Arthur L. Burnett, M.D., M.B.A., F.A.C.S.**

Johns Hopkins University School of Medicine

**Dr. Arthur (Bud) Burnett** received his A.B. degree in Biology from Princeton University and his M.D. and M.B.A. degrees from Johns Hopkins University. He performed his post-graduate training in general surgery, urology, and reconstructive urology and urodynamics at the Johns Hopkins Hospital. He received an American Foundation for Urologic Disease scholarship and joined the faculty at the Johns Hopkins University School of Medicine, currently serving as the Patrick C. Walsh Distinguished Professor of Urology. He is the Director of the Basic Science Laboratory in Neuro-urology of the James Buchanan Brady Urological Institute and Director of the Sexual Medicine Division and Fellowship Program in the Department of Urology, Johns Hopkins Medical Institutions. He is an alumni member of the Alpha Omega Alpha Honor Medical Society and Fellow of the American College of Surgeons.

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**Nitric Oxide Function and Dysregulation in the Lower Genitourinary Tract: Health and Disease**

The neurobiology of the pelvis has had a rich tradition of discoveries spanning from neuroanatomy to neurochemistry of the pelvic region, which have served to shape currently accepted views of the neuroregulatory basis of pelvic functions. Within this discipline, current thinking about the regulatory mechanisms of autonomic function in the genitourinary system and pelvis that involves local neurotransmitters has recognized such extraordinary roles of gaseous signaling molecules. Gaseous molecules such as nitric oxide (NO) represent a relatively new class of neurotransmitters and mediators operating through unconventional signal transduction pathways with profound impacts on pelvic function.

The role and actions of NO are well associated with erection physiology. The chemical is characterized as the principal mediator of penile erection, functioning to induce corporal smooth muscle relaxation required for penile engorgement. The NO signaling pathway is also understood to conduct a major homeostatic regulatory function in the penis and participate in maintaining a steady state vascular biologic purpose in this organ. These interactions include transnitrosylation, post-translational modifications (e.g., phosphorylation), oxidative/nitrosative stress regulation, and co-regulatory molecular expression and function (e.g., RhoA/Rho-kinase, phosphodiesterase type 5). Derangements in tonic NO signaling (i.e., NO imbalance) in the penis are now understood to explain such pathologic erection disorders as recurrent ischemic priapism, a clinically vexatious phenomenon of prolonged and uncontrolled penile erection.

New research surrounding NO signaling has suggested this molecule exerts homeostatic roles in other locations of the lower genitourinary tract besides the penis and thereby may contribute to the regulation of urinary continence and micturition. This role is distinct from the previous descriptions of its NO agency in tissue relaxation and sensory regulation of the in the lower urinary tract; rather its homeostatic role refers to NO signaling dysregulation that may directly account for urinary abnormalities such as detrusor overactivity.

This presentation will principally cover the evolution of research work describing NO in the physiology of penile erection and the pathophysiology of erection disorders and also explore NO dysregulation as a common pathomechanism for lower genitourinary tract dysfunction.
Dr. Bavendam joined the National Institute of Diabetes and Digestive and Kidney Diseases in October 2012 as a Senior Advisor for Women’s Urologic Health. As a urologist, the focus of her career has been to reduce the impact of lower urinary tract conditions on the lives of women. Dr. Bavendam received her training at the University of Iowa. She was a full-time faculty at the University of Washington in Seattle and Drexel University in Philadelphia. After 20 years of direct patient care, Dr. Bavendam joined Pfizer where she was involved in the clinical trial programs for sexual dysfunction and overactive bladder. At the NIDDK Tamara is building a portfolio of investigator initiated research in all aspects of women’s urologic health and conditions. She is a project officer for the Prevention of Lower Urinary Tract Symptoms (PLUS) Research Consortium launched in 2015. The PLUS Consortium will identify and test promising strategies for promoting bladder health and preventing lower urinary tract conditions in women throughout their lifespan using both qualitative and quantitative research. The focus will be on obtaining the necessary information to plan future educational efforts to promote bladder health and prevention of conditions associated with LUTS such as urinary incontinence, urologic chronic pelvic pain syndrome and interstitial cystitis.

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Opportunities for Funding Cross-Disciplinary Pelvic Research

Dr. Bavendam will describe the opportunities for Pelvic Research funding through NIH.
Dr. Grannum R. Sant, MD, FRCS, FACS
Tufts University School of Medicine

**Dr. Sant** is currently a Professor of Urology, Tufts University School of Medicine and the Principal/Founder Sant Consulting LLC. He is a former Chair of Urology, Tufts University School of Medicine, Boston. His industry experience includes being the Head of US Medical Affairs (Oncology and Urology) for Sanofi US and Head of Global Medical Affairs (Rare Diseases) for Genzyme. Dr. Sant led the medical affairs launch of 7 therapeutic products including Taxotere and Jevtana for metastatic prostate cancer, Eligard for prostate cancer and Uroxatral for BPH. He also developed of the O’Leary-Sant patient – reported outcomes for interstitial cystitis (IC).

He is an Editorial Board Member of 7 journals including Canadian Journal of Urology and Translational Andrology and Urology. He is the Editor of 10 Urology Journal Supplements and 2 textbooks of urology and author of over 120 peer-reviewed publications.

Currently Founder and Principal of Sant Consulting LLC – offering mentoring and strategic advice to individuals and companies in the biopharmaceutical industry in the therapeutic areas of urology, oncology and rare diseases.

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**Transition from Academia to Industry - Pathway and Opportunities**

There is a significant career advancement roadblock for post-docs and PhDs in academia with less that 10% gaining academic tenure.

The biopharmaceutical industry offers opportunities for transition from academia that are rewarding from both the financial and quality-of-life perspectives. I will apply lessons learnt from my own transition from academia to industry to inform PhDs and MDs in academia of opportunities in the biopharmaceutical industry.

The biotech and pharma industry offers financial and quality of life appeal to post-docs and junior academic faculty in the life science and biologic/life sciences. The lecture will offer do’s and don’t’s for individuals considering a transition from academia to industry.
**Title:** Mechanosensitive release of ATP and other purines from the bladder urothelium  

**Authors:** Violeta N. Mutafova-Yambolieva* and Leonie Durnin  

**Affiliations:** Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, Nevada, USA  

**Introduction/Objectives:** Adenosine 5’-triphosphate (ATP) is released from the bladder mucosa during bladder filling and is assumed to stimulate sensory neurons in the lamina propria and to regulate the apical delivery of fusiform vesicles in urothelial umbrella cells. As a parasympathetic cotransmitter, ATP contracts the detrusor smooth muscle. Therefore, extracellular ATP likely regulates the two phases of the micturition cycle (i.e., bladder filling and emptying) by different mechanisms. We recently demonstrated intraluminal release of purines in addition to ATP during bladder filling. The present study was designed to expand on previous works and evaluate simultaneously the release of ATP, ADP, AMP, nicotinamide adenine dinucleotide (NAD+)/ADP-ribose and adenosine from both sides of the urothelium during bladder filling.  

**Methods:** The luciferin-luciferase assay method that is commonly used to evaluate ATP release in the bladder overlooks release of biologically-active purines that are different from ATP. Urothelial release of ATP is frequently studied in isolated bladder wall sheets mounted in Ussing chambers, which does not mimic typical bladder filling. In contrast, we determined release of purines in whole decentralized (ex vivo) mouse (C57/BL6) bladder preparations with removed detrusor smooth muscle that were filled with either 50 ml (low volume) or 200 ml (high volume) physiological solution at 15 ml/min. After distention, both extraluminal (bath fluid aliquots) and intraluminal fluids were collected and preserved with citric buffer (pH 4.0). The presence of multiple purines was simultaneously evaluated by ultra-sensitive HPLC-FLD methodologies.  

**Results:** ATP, ADP, AMP, NAD+/ADPR and adenosine were detected intraluminally and extraluminally, suggesting that cells in the bladder mucosa and the suburothelium/lamina propria are likely exposed to a variety of purines during bladder filling. Since the amounts of detected purines at any particular time represent released minus removed purines, greater degradation likely occurs during the longer bladder filling with 200 ml fluid. Yet, higher purine amounts were generally detected, on both sides, at 200 ml filling volume. The rate of purine metabolism during different stages of bladder filling remains to be determined. The ratio of intraluminal to extraluminal content differed for individual purines. Thus, the intraluminal ATP was ~10-folds higher than the extraluminal ATP at both filling volumes, whereas the intraluminal NAD+ exceeded the extraluminal NAD+ ~5-folds and adenosine amounts were similar on both sides of the urothelium.  

**Conclusions:** Multiple purines are released from both sides of the mucosa during bladder filling, possibly stimulating multiple cell type targets in the bladder wall. It remains to be determined whether intraluminally-released purines can be transported to the suburothelium or reach the detrusor smooth muscle. Further studies are warranted to understand the mechanisms of mechanosensitive release, transport and metabolism of purines in the bladder wall and the role of these purines in regulating bladder functions during the micturition cycle.  

**Funding Source:** NIH grant DK 41315
Title: Influence of Mucosal Remodeling on Urothelial Functions

Authors: Lori Birder, PhD

Affiliations: University of Pittsburgh

Introduction/Objectives: Lower urinary tract symptoms (LUTS), in particular storage symptoms are a major health related problem. Much of research past and present, has focused on detrusor muscle function and changes in the central neurological control of LUT function; however, much less is known about the role of the urothelium and cells within the lamina propria in these events. Our research has shown that decreased vascular perfusion and changes within the extracellular matrix (ECM) can significantly impact urothelial signaling.

Methods: These include microangiography (for vascular imaging) as well as concurrent (multi-photon) imaging and mechanical testing of collagen fibers in the bladder wall of the rat.

Results and Conclusions: Besides sensing chemical signals, the urothelium is highly responsive to mechanical loads thus the mechanical properties of the lamina propria and associated ECM may an important role in its function. Thus, changes in release of mediators and fiber architecture with age or bladder pathology can adversely impact the mechanobiology and hence sensation and bladder function.

Funding Source: NIH
**Title:** In vitro evaluation of hydrostatic pressure on ATP release and Caspase-1 activation in rat urothelial cells

**Authors:** Cody L. Dunton\(^1\)*, F. Monty Hughes \(^{1,2}\), J. Todd Purves \(^{1,2}\), Jiro Nagatomi\(^1\)

**Affiliations:** \(^1\)Department of Bioengineering, Clemson University, Clemson, SC, USA, \(^2\)Department of Urology, Duke University, Durham, NC, USA

**Introduction/Objectives:** Bladder outlet obstruction (BOO) is projected to affect approximately 1.1 billion men and women by 2018.\(^4\) While BOO results from a multitude of etiologies, they are all characterized by chronically elevated storage and voiding pressures, along with inflammation in the bladder tissue, which can lead to bladder fibrosis and overactive bladder symptoms.\(^1\) Previous research in our lab demonstrated that ATP release by rat primary urothelial cells increased in response to elevated pressure (10-15 cmH\(_2\)O) in vitro.\(^3,5\) Thus, we hypothesize that the release of ATP by urothelial cells upon exposure to pathological pressures in BOO initiates a cascade of events that includes the formation of the NLRP3 inflammasome and caspase-1 activation. In the present study, we examined the effects of elevated pressure on ATP-purinergic signaling and caspase-1 activation in rat urothelial cells in vitro.

**Methods:** Using a custom built pressure system\(^5\), rat urothelial cell line MYP3 cells (1.2\(\times\)10\(^6\) cells/well in F-12 K medium) were exposed to pressure conditions that represent both pathological storage and voiding pressures: 15 cmH\(_2\)O 60 min, 15 cmH\(_2\)O 1 min, 40 cmH\(_2\)O 1 min, and 75 cmH\(_2\)O 1 min. Cells that were prepared in a similar manner but maintained under atmospheric pressure served as a control. In addition, MYP3 cells that were exposed to a hypotonic condition (240 mOsm) and cells treated with 1.25 mM ATP served as two positive controls. After exposure to pressure, the supernatant media was collected and the extracellular ATP concentration was measured using a luciferin-luciferase assay kit (Life Technologies). Cells were then lysed and intracellular caspase-1 activity was measured using an established method.\(^2\) The pressure experiments (40 cmH\(_2\)O 1 min) were repeated in the presence of a P2X7 antagonist (100 µM A-438079 (R&D Systems)) to determine the mechanism for pressure-induced caspase-1 activation.

**Results:** Exposure of MYP3 cells to hydrostatic pressure for 1 min at 15 cmH\(_2\)O, 40 cmH\(_2\)O, and 75 cmH\(_2\)O resulted in a 3 fold, 3.5 fold, and 4.5 fold increase in extracellular ATP levels, respectively, compared to the 0 cmH\(_2\)O control. When these cells were exposed to a hypotonic solution, ATP release increased by 6-fold compared to the isotonic control. Exposure of MYP3 cells to pressure also resulted in up to 1.3 fold increases in caspase-1 levels, which were similar to the positive controls, indicating caspase-1 levels are maximized after exposure to pathologic pressure conditions.

**Conclusions:** The significant increase in ATP release indicates that hydrostatic pressure is a good mechanical stimulus to model BOO in vitro. The acute caspase-1 responses after 1-minute exposure to pressure suggest that high-pressure voiding may be an important trigger of the NLRP3 inflammasome in BOO. The results that demonstrate exposure to pressure and treatment with ATP both result in caspase-1 activation implicate autocrine purinergic signaling as a mediator of NLRP inflammasome formation.

**Funding Sources:** NIH (R01DK103534, P20GM103444), NSF (1264579)
Title: Myosalpinx Contractions are Essential for Egg/Embryo Transport in the Oviduct

Authors: Sean M. Ward

Affiliations: Department of Physiology, University of Nevada, Reno School of Medicine, Reno Nevada, USA.

Introduction: Oviducts or fallopian tubes are smooth muscle lined dynamic tubular organs that connect the ovaries to the uterus and facilitate several physiological processes including gamete transport, fertilization and early embryo development. Several stages of the reproductive process occur exclusively within the oviduct and are subject to physiological regulation. The four main functions of the oviduct include: (i) The transport of the ovum from the ovary to the site of fertilization. (ii) The oviduct aids in transport of spermatozoa from the site of deposition to the site of fertilization within the ampulla. (iii) The oviduct provides a suitable environment for the egg with secretions along the duct providing protection of the egg from mechanical damage and for maintenance of viability of the egg during its transport. (iv) Transportation of the fertilized ovum (embryo) to the uterus where implantation and further development occurs.

Objectives: Given the complexities and the different physiological functions of the oviduct it is not surprising that it is functionally organized into different regions. Dogma in the literature suggests that egg or embryo transport depends mainly on the incessant beat of the ciliated epithelium towards the uterus. However, egg transport may also be dependent upon contractions of the smooth muscle lined wall or myosalpinx. In order to fully understand the physical mechanisms that contribute to the coordinated movement of the egg we investigated the role of the calcium-activated chloride conductance, Ano1 on the generation of pacemaker activity.

Methods: Video imaging and spatio-temporal mapping along with intracellular microelectrode recordings were performed to define the role of myosalpinx in egg/embryo transport. The Cre-recombinase LoxP technology was also utilized to determine the role of the calcium-activated chloride conductance, Ano1 in adult tissues.

Results: Egg transport is highly dependent upon phasic contractions of the smooth muscle lined wall or myosalpinx. The phasic and propulsive contractions of the myosalpinx depend upon specialized interstitial cells of Cajal, termed ICC-OVI. There is regional dependence on the role of Ano1 in pacemaker activity and that global reduction in Ano1 in mature female mice leads to reduced fertility.

Conclusions: These data further support the role of myosalpinx activity in egg/embryo transport in the oviduct and the importance of Ano1 in the generation of pacemaker activity within the oviduct. A critical role in Ano1 in egg/embryo transport and fertility is also demonstrated.

Funding Source: NIH RO1 DK57236
Title: Purinergic regulation of nitric oxide mediated vasodilation in rat internal pudendal arteries

Authors: Michael R. Odom¹*, Johanna L. Hannan¹

Affiliations: ¹Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, USA.

Introduction/Objectives: Internal pudendal arteries (IPA) provide 70% of total blood flow resistance to penile arteries and have been shown to undergo significant vascular remodeling due to aging, diabetes, chronic kidney disease, and cardiovascular disease. Balloon angioplasty and stent placement is a common treatment option to restore blood flow within IPAs; however, stent failure is disproportionately higher than coronary artery stents despite similar stents and placement techniques suggesting a unique IPA physiology. Non-adrenergic non-cholinergic (NANC) mediated vasodilation is well characterized in the penis, but IPA neurotransmitter release has yet to be characterized. The objective of this study is to characterize electric field stimulated (EFS) mediated neurotransmitter release in rat IPA and address the novel role of ATP as a neurotransmitter.

Methods: Proximal (internal iliac to gluteal artery) and distal (gluteal artery to penis) IPA segments excised from male Sprague-Dawley rats (350-475g) were mounted into myographs. NANC vasodilation was assessed in precontracted IPAs following incubation with inhibitors of norepinephrine release (guanethidine; 10⁻⁶M) and cholinergic receptors (atropine, 10⁻⁵M) to assess neuronal nitric oxide (NO) release. This procedure was repeated with additional incubation of a NO synthase (NOS) inhibitor (L-NAME; 10⁻⁶M), selective P2X1 inhibitor (NF-449; 10⁻⁵M), or selective P2Y1 inhibitor (MRS 2500; 10⁻⁶M). EFS relaxation parameters used were 5ms delay, 0.3ms duration at 20V and 0.5-32 Hz. Concentration response curves to assess ADP, a P2Y1 receptor agonist, relaxation and α,ß-MetATP, a stable P2X1 receptor agonist, contraction were also performed.

Results: NANC stimulation lead to greater relaxation in distal IPA (30% vs 15%, p<0.05). Additional inhibition of NOS completely eliminated NANC relaxation in distal IPA; however, did not reduce proximal IPA relaxation. P2X1 or P2Y1 inhibition caused further distal IPA relaxation, but combined P2X1 and P2Y1 inhibition increased distal IPA relaxation from 30% to 53% without affecting proximal IPA relaxation. No significant differences were noted to α,ß-MetATP mediated contractions via the P2X1 receptor. ADP mediated relaxation was markedly increased in proximal IPA (16%) compared to distal IPA (6%).

Conclusions: Functional physiological differences exist within the proximal and distal segments of the IPA. NANC-mediated relaxation in the distal IPA is greater than the proximal IPA and is mediated by NO-dependent mechanisms. Furthermore, the distal IPA undergoes additional purinergic regulation which is NO-independent. Understanding the physiology of this critical feeder blood vessel to the penis is crucial when developing novel treatments that target the vasculature to recover erectile function.

Funding Source: None.
Title: Fibrosis in the bladder in response to outlet obstruction is triggered through the NLRP3 inflammasome and the production of IL-1β

Authors: Francis M. Hughes, Jr., Vihasa Govada, Stephanie Sexton and J. Todd Purves

Affiliations: Department of Surgery, Division of Urology, Duke University Medical Center, Durham, NC.

Introduction/Objectives: Bladder outlet obstruction (BOO), most commonly created by benign prostatic hyperplasia, promotes an inflammatory state that produces voiding dysfunction. Recently, we have shown that the NLRP3 inflammasome triggers this inflammation. Over time, inflammation promotes fibrosis. In this study we explore the role of NLRP3 (and IL-1β produced by activated NLRP3) in BOO-induced fibrosis.

Methods: Rats were divided into 5 groups: 1) control, 2) sham, 3) BOO + Vehicle (Veh; 1 ml, 40% EtOH, p.o.), 4) BOO + glyburide (Gly; NLRP3 inhibitor; 10 mg/kg, p.o.) or 5) BOO + Anakinra (Ana; IL-1 receptor antagonist; 25 mg/kg, i.p.). BOO is constructed by inserting a 1 mm transurethral catheter, tying a suture around the urethra and removing the catheter. Medications were given prior to surgery and once daily for 12 days. Hypertrophy was assessed by bladder weight, fibrosis by collagen staining (Masson’s Trichrome Stain), IL-1 receptor 1 (IL-1R1), prolyl 4-hydroxylase (P4H) and lysyl oxidase (LOX) localization by immunofluorescence and collagen secretion by Sirius Red.

Results: BOO increased production of collagen in the bladder which was blocked by either preventing NLRP3 activation with glyburide or blocking IL-1β’s action at its receptor, clearly implicating the NLRP3/IL-1β pathway in fibrosis during BOO. IL-1β directly triggers collagen synthesis in other tissues and we found that recombinant IL-1β stimulated pro-collagen production in control urothelial cells placed in culture. Consistent with urothelia as a source of pro-collagen production, isolated urothelial cells from BOO rats secreted significantly more IL-1β than control cells. In control rats, the IL-1β receptor, IL-1R1, was highly expressed in the basal layer of the urothelia with less staining in the umbrella cells and detrusor and no staining in the interstitium. P4H (a marker of pro-collagen synthesis) exhibited similar staining, although enhanced expression in the basal urothelia was not as prominent. LOX (marker of mature collagen fibril production) was also found in urothelia and detrusor but additionally in the interstitium (the site of mature collagen fibril production). In BOO rats, the hyperplastic urothelia contained multiple layers of cells that all expressed IL-1R1 with P4H and LOX. Gly and Ana prevented this change in IL-1R1 distribution.

Conclusions: NLRP3-derived IL-1β triggers fibrosis during BOO, most likely through an autocrine loop in which IL-1β acts back on urothelia to drive collagen production.

Funding Source: NIDDK: R01DK103534 (PI - Purves)
S1A7 was withdrawn
Title: Altered S-Nitrosation of Contractile Proteins Underlies Dysfunctional Quiescence in Human Preterm Labor

Authors: Iain L. O. Buxton, Craig Ulrich, Scott D. Barnett, and Heather R. Burkin

Institutional Affiliation: Department of Pharmacology, University of Nevada School of Medicine, Reno, Nevada, United States, 89557. Email: ibuxton@medicine.nevada.edu

Objectives: Preterm labor is defined as labor prior to 37 weeks of gestation. There are no FDA approved drugs available to treat spontaneous preterm labor. Uterine smooth muscle is unique in that it relaxes in a cGMP-independent manner in the presence of nitric oxide (NO). However, NO-mediated relaxation in tissues from mothers who deliver preterm is blunted. S-nitrosation of cysteines via NO acts as an important mediator in a number of disease states. Our analysis of the myometrial S-nitrosoproteome has revealed that several smooth muscle contractile proteins are differentially S-nitrosated based upon the state of labor in women. An important regulator of protein S-nitrosation is the availability of S-nitrosoglutathione (GSNO), an endogenously expressed NO donor. The enzyme GSNO reductase (GSNOR) regulates GSNO levels in smooth muscle. We performed experiments to test the hypotheses that GSNOR activity is altered in the myometrium from women in preterm labor and that inhibition of GSNOR attenuates myometrial contraction and restores quiescence in spontaneous preterm labor.

Methods: Experiments were performed in the Myometrial Research Laboratory at UNR Med. Uterine biopsies were obtained with IRB approval and written informed consent from mothers undergoing Cesarean section and transported to the laboratory in physiological buffer for immediate processing. GSNOR expression was measured by Western blot and normalized to GAPDH expression. GSNOR activity was measured by the decrease in absorbance at 340nm via conversion of NADH to NAD⁺ by GSNOR in the presence of GSNO. Eight (8)nM N6022, a potent and selective inhibitor of GSNOR, was used to verify GSNOR specific activity. Human myometrial tissue was dissected and six uterine strips from each patient were mounted in tissue baths and tested for their ability to contract in response to KCl prior to addition of test agents.

Results: Human preterm myometrium fails to relax to NO addition whether in the presence or absence of oxytocin stimulation. GSNOR expression is increased in myometrial tissue from women in spontaneous preterm labor compared to term laboring controls. GSNOR enzymatic activity was also increased in protein lysates from myometrial tissue from patients undergoing spontaneous preterm labor than those in labor at term. A potent and selective inhibitor of GSNOR (N6022) decreases the peak force of contraction in guinea pig myometrium and restores sensitivity to NO.

Conclusion: GSNOR is more highly expressed in preterm laboring myometrium. Preterm laboring myometrium exhibits a blunted response to NO and the GSNOR inhibitor N6022 restores sensitivity to relaxation by NO. Our data suggests that increased GSNOR activity in spontaneous preterm labor contributes to a preterm contractile phenotype through the enzymatic degradation of endogenous GSNO.

Funding Source: This research was supported by NIH 1U54GM 104944.
Title: A method to quantify bladder wall biomechanics using ultrasound imaging in conjunction with urodynamics

Authors: Anna S. Nagle¹, Adam P. Klausner², Jary Varghese³, Rachel Bernardo⁴, Andrew F. Colhoun², Paul H. Ratz⁵, R. Wayne Barbee⁶, Laura R. Carucci³, John E. Speich¹

Affiliations: ¹Department of Mechanical & Nuclear Engineering, Virginia Commonwealth University School of Engineering, Richmond, VA. ²Department of Surgery/Division of Urology, Virginia Commonwealth University School of Medicine, Richmond, VA. ³Department of Radiology, Virginia Commonwealth University School of Medicine, Richmond, VA. ⁴Department of Biomedical Engineering, Virginia Commonwealth University School of Engineering, Richmond, VA. ⁵Department of Biochemistry & Molecular Biology, Virginia Commonwealth University School of Medicine, Richmond, VA. ⁶Department of Emergency Medicine, Virginia Commonwealth University School of Medicine, Richmond, VA

Introduction/Objectives: Overactive bladder (OAB) is often associated with detrusor over-activity which is characterized by biomechanical changes in the smooth muscle of the bladder wall, but there is no established method to measure these changes in vivo. This study’s goal was to develop a novel method to determine detrusor wall biomechanical parameters during urodynamics through the incorporation of abdominal ultrasound imaging.

Methods: Individuals with OAB underwent ultrasound imaging during filling. Fill rate was 10% cystometric capacity (CCap) as determined by an initial fill. Ultrasound images were obtained using a Philips Epiq 7 machine with a 1-5 MHz abdominal probe to capture midsagittal and transverse images at 1 min intervals. Using image data and vesical pressure (Pves), detrusor wall tension, stress, and compliance were calculated. From each cross-sectional image, luminal and wall areas along with inner perimeters were measured. In the sagittal and transverse planes, wall tension was calculated as Pves*luminal area, wall stress as tension/wall area, and strain as the change in perimeter normalized to the perimeter at 10%CCap. Elastic modulus was calculated as stress/strain in each direction. Patient-reported fullness sensation was continuously recorded.

Results: Data from five individuals with OAB showed that detrusor wall tension, volume, and strain had the highest correlation to continuous bladder sensation of all quantities measured. This finding demonstrates that Pves and Pdet measurements during urodynamics may not necessarily reflect the underlying state of detrusor wall tension.

Conclusions: This study demonstrates that detrusor wall tension, stress, strain, and elastic modulus can be calculated by adding ultrasound imaging to standard urodynamics. This technique may be useful in better understanding the biomechanics involved in OAB and other bladder disorders.

Funding Sources: Research funding for this study was provided by the Virginia Commonwealth University Presidential Research Quest Fund and NIH grant R01DK101719.
Title: Down-regulation of ryanodine receptor gene expression in murine urinary bladder smooth muscle following partial bladder outlet obstruction

Authors: Etticken Boopathi*1, Elham Javed1, Shankar Addya2, Paolo Fortina2, Stephen Zderic3, Alan Wein4, and Samuel Chacko5

Affiliations: 1Department of Medicine, 2Kimmel Cancer Center, Thomas Jefferson University, 3Division of Urology, Children’s Hospital of Philadelphia, 4Division of Urology, 5Department of Pathobiology, University of Pennsylvania, Philadelphia, PA

Introduction/Objectives: Urinary bladder smooth muscle (UBSM) displays spontaneous action potentials and this potential is related to the phasic nature of spontaneous contractions in this tissue. The amplitude of a phasic contraction depends on the increase in Ca²⁺ entry caused by membrane depolarization. Ryanodine receptors (RyRs) in UBSM decreases the force production by decreasing the frequency of phasic contractions through interactions with large-conductance Ca²⁺-activated K⁺ (BK) and small-conductance Ca²⁺-activated K⁺ (SK) channels. Microarray and network analysis were employed to determine the changes in mRNA in 14-day obstructed murine bladders. We found that obstruction significantly down-regulated the RyRs in bladder smooth muscle (BSM).

Methods: Male C57Bl/6 mice were surgically obstructed and kept for 14 days. Sham-operated mice served as a control. Bladders were excised; urothelium scraped off with a scalp, and the serosa was removed. BSM obtained from PBOO and sham control animals were used for microarray and western blotting.

Results: Pathway-based analysis of these gene signatures showed significant number of under-expressed genes in obstructed bladder and they were mapped to proteins involved in calcium signaling. We focused our work on RyR protein expression in BSM. There was a four-fold reduction of RyR3 in BSM in 14-day obstructed groups as shown by microarray and immunoblotting compared to that of sham-operated animals.

Conclusion: These results confirm that the RyR gene expression is down-regulated in obstructed murine bladder smooth muscle.

Funding Source: None
Title: What’s Wrong with UAB Patients?

Authors: Phillip P. Smith MD1,3,4, Gerard Pregenzer MD2, Andrew Boylen MD1, Jason Frankel MD1, George Kuchel MD3

Affiliations: 1Department of Surgery, Division of Urology, University of Connecticut School of Medicine, Farmington CT; 2St Francis Hospital, Hartford CT; 3Center on Aging, University of Connecticut School of Medicine, Farmington CT; 4UConn Institute for Brain and Cognitive Science, University of Connecticut, Storrs CT

Introduction/Objectives: Underactive Bladder (UAB) is receiving increased attention as a contributor to urinary bother. An emerging definition of UAB focuses on voiding symptoms suggestive of urodynamic detrusor underactivity. However, while animal and tissue studies are useful to elucidate pathophysiological mechanisms, they cannot directly address human symptoms. Objective dysfunctions associated with UAB symptoms remain unclear. We therefore sought to describe the urodynamic observations associated with voiding symptoms consistent with UAB.

Methods: A retrospective review of 500 consecutive urodynamic studies conducted by one urodynamicist in one clinical laboratory. Charts of patients with known neurologic disease, prior bladder or urethral surgery, and charts lacking symptom descriptions were excluded. Age, sex, primary and secondary symptoms, urodynamic variables and observations (UO) were logged. 3 groups were identified for analysis and comparison: Primarily UAB symptoms (UAB1), any UAB symptoms (UAB), and no UAB symptoms.

Results: 421 charts were suitable for abstraction. Of 132 patients with any UAB, 100 were UAB1. Of the remainder 16 were primarily storage/OAB symptoms, and 14 were primarily incontinence. Average age of UAB patients was 56+-1.5 yrs, vs. 57.4+-1.0 for non-UAB (n.s.). UAB1 patients were 59.1+-1.8 yrs old vs. 54.7+-2.4 yrs for UAB (n.s.). For UAB1 and UAB, BOO was the most common UO, 35/40% of patients having outlet obstruction (BOO), 25/25% had detrusor underactivity (DU), 20/26% were dysfunctional voiders (DV), 12/13% had detrusor overactivity, 5/8% had SUI, and 3/5% had volume hypersensitivity. UAB1 was more prevalent in men, 54m/46f, however overall 76 females vs. 56 males had any UAB. DU was more common in women than men (21f/12m) with UAB, but BOO was more common in men (37m/13f). Overall, UAB had higher volume sensations thresholds than non-UAB (p<0.05), however less consistently for UAB1 and for UAB patients with BOO, DU, or dysfunctional voiding. Watts Factor did not differ by UAB, BOO, or DU, but PVR was greater in UAB1, UAB, BOO and DV patients with UAB vs. nonUAB patients (p<0.05). For DU, PVR in UAB patients was 376+-50 vs. 255+-37ml for nonUAB, p=0.06. Patients with BOO and DU were older, but did not differ by age according to the presence/absence of UAB.

Conclusions: Urodynamic observations related to impaired voiding (BOO, DU, DV) but not impaired contractility account for most urodynamic dysfunctions in UAB patients. An elevated PVR and diminished volume sensations contribute to the occurrence of UAB. Animal models of UAB should address the reasons for diminished system sensitivity to bladder volume rather than a limited focus on detrusor motor deficiencies.

Funding Source: None
Title: Evidence supporting a pivotal role for intramuscular interstitial cells of Cajal in the generation of pacemaker activity, phasic contractions and tone in the internal anal sphincter.

Authors: Kathleen D. Keef, Sean M Ward and Caroline A. Cobine

Affiliations: Department of Physiology and Cell Biology, University of Nevada, Reno School of Medicine, Reno Nevada, USA.

Introduction/Objectives: A fundamental property of the internal anal sphincter (IAS) is its ability to generate tone. However, the mechanism underlying tone generation is still controversial. In other GI regions, interstitial cells of Cajal (ICC) have been demonstrated to be the pacemaker cells that generate slow waves (SWs). We have shown that SWs are also present in the IAS along with a population of intramuscular ICC (ICC-IM). However, the identity of the cell that generates SWs in the IAS is still uncertain. We hypothesize that SWs are generated by ICC-IM via a rise in intracellular calcium, activation of calcium-activated chloride channels (ANO1) and voltage-dependent L-type calcium channels (CavL). We further propose that SWs play a critical role in tone development in the IAS through the generation and summation of phasic contractile activity.

Methods: Cell specific protein expression was examined with immunohistochemistry while gene expression was determined with qPCR on whole muscles and cells isolated with FACS. An inducible Cre/loxP technique was used to express a genetically-encoded Ca²⁺ biosensor (GCaMP3) in a cell-specific manner. Calcium transients were imaged from the IAS of mice expressing GCaMP3 in ICC (Kit-GCaMP3) and smooth muscle cells (SMC; smMHC-GCaMP3) with an Olympus DSU.

Results: Dual labeling immunohistochemistry revealed ANO1 expression in ICC but not SMC while gene expression of ANO1 (Ano1) was 26x greater in FACS-sorted ICC than in sorted SMC. In contrast, gene expression of CavL (Cacna1c) was only 2x greater in SMC than in ICC. Calcium transients were visualized in Kit-GCaMP3⁺ cells in the circular muscle layer of the IAS. Kit-GCaMP3⁺ cells exhibited rhythmic whole cell Ca²⁺ transients that occurred at the same frequency as the SWs we have previously recorded from this muscle. The activity of adjacent Kit-GCaMP3⁺ cells (<200 µm) was synchronized suggesting coupling between ICC-IM. Rhythmic calcium transients in Kit-GCaMP3⁺ cells were abolished by removal of extracellular calcium, by the CavL blocker nicardipine (1 µM) and the ANO1 blocker CaCCinh-A01 (3-10 µM). SWs and phasic contractions were also abolished by these blockers. Like SWs, Ca²⁺ transients in both smMHC-GCaMP3⁺ and Kit-GCaMP3⁺ cells were greatest in frequency at the distal extremity of the IAS and declined in the proximal direction. However, when the distal edge of the IAS was removed, both SWs and Ca²⁺ transients persisted in the remaining muscle although there was a reduction in frequency.

Summary and Conclusions: This study provides the first direct evidence that ICC-IM are the pacemaker cells of the IAS. The properties of Ca²⁺ transients in Kit-GCaMP3⁺ and smMHC-GCaMP3⁺ cells are commensurate with the electrical and contractile properties of this muscle. The ability of nicardipine and CaCCinh-A01 to abolish tone, SWs and rhythmic calcium transients in Kit-GCaMP3⁺ cells suggests a causal relationship between these events. The importance of the distal edge of the IAS in regulating phasic contractions in this muscle is underscored by the decline in both SW and Ca²⁺ transient frequency and amplitude in the proximal direction indicating that like the heart there is a dominant site of pacemaker activity in the IAS. The observation that the greatest frequency of these events is at the distal edge suggests an important function in ensuring fecal continence.

Funding Source: NIH DK078736.
Title: Sex differences and participation of Toll-like receptor 4 to rat bladder contractile function

Authors: Theodora Szasz, Beth Burgess, R. Clinton Webb

Affiliations: Department of Physiology, Augusta University, Augusta, GA, USA

Introduction/Objectives: Innate immune mechanisms have been implicated in the pathophysiology of chronic sterile conditions such as hypertension and diabetes. We have recently demonstrated that Toll-like receptor 4 (TLR4) activation by endogenous molecules such as high mobility group box-1 (HMGB1) contributes to hypertrophy and hypercontractility in diabetic bladder dysfunction. It has been reported that women have a higher frequency of overactive bladder symptoms, while men have higher detrusor overactivity. We hypothesized that sex differences in the contribution of TLR4 to bladder contraction may underlie the sex differences observed clinically.

Methods: Female and male rat bladder contractile responses to carbacholine (CCh) and electrical field stimulation (EFS) were measured in the presence and absence of TLR4 inhibitor CLI-095 and in the presence and absence of urothelium.

Results: We observed that contractile responses to both CCh and EFS were higher in the male than the female bladder segments in both the presence and absence of urothelium (CCh Emax (mN): male+urothelium = 84.3±1.2, male-urothelium = 83.7±1.2, female+urothelium = 49.8±0.8, female-urothelium = 59.2±1.1; EFS 32 Hz (mN): male+urothelium = 84.9±7.1, male-urothelium = 66.8±5.2, female+urothelium = 57.8±5.9, female-urothelium = 54.5±3.5). Incubation of bladder segments with the TLR4 inhibitor CLI-095 significantly decreased contractile responses to both CCh and EFS in both sexes, irrespective of the presence of urothelium (CCh Emax (mN): male+urothelium = 91.1±0.8, male-urothelium = 75.4±1.8, female+urothelium = 42.9±1.1, female-urothelium = 52.5±1.1; EFS 32 Hz (mN): male+urothelium = 80.8±7.9, male-urothelium = 59.6±10.6, female+urothelium = 43.3±2.6, female-urothelium = 46.4±1.9).

Conclusions: Our data suggest that although there are sex differences in the contractile function of the rat bladder in basal conditions, the participation of TLR4 to bladder contraction in the absence of endogenous activators is similar between the two sexes.

Funding Source: NIDDK Diabetic Complications Consortium (DiaComp, www.diacomp.org), grant DK076169.
Title: High fat diet leads to impaired mitochondrial respiration, increased hydrogen peroxide emission and greater nerve-mediated purinergic contraction in mouse bladder

Authors: Shelby A. Powers,1* Terence E. Ryan,1,2 Joseph M. McClung,1,2 Johanna L. Hannan1,2

Affiliations: 1Department of Physiology; 2East Carolina Diabetes and Obesity Institute, Brody School of Medicine, East Carolina University, Greenville, NC, USA

Introduction/Objectives: Chronic high fat diet (HFD) is associated with the development of metabolic syndrome and type 2 diabetes, known risk factors for lower urinary tract symptoms, overactive bladder, and incontinence. HFD induced increases in oxidative stress and impaired mitochondrial integrity is a possible mechanism underlying this pathology. Our objectives were to determine bladder function following long-term HFD and characterize mitochondrial oxidant emission and respiratory function. We hypothesized that chronic HFD would lead to increased cholinergic mediated contraction and impaired mitochondrial respiration.

Methods: Male C57BL/6J mice were maintained on a control diet (CD: 4% fat, 60% carbohydrates, 26% protein) or a HFD (45% fat, 35% carbohydrates, 20% protein) from 6 weeks of age for 20 weeks. A week prior to sacrifice, lower urinary tract function was assessed via void spot assay. Animals were euthanized and bladders and visceral fat depots were collected and weighed. Bladders were denuded of urothelium, cut into 8x2mm strips and placed in tissue baths. Increasing concentrations of carbachol (cholinergic mediated) contraction and adrenergic relaxation to norepinephrine (NE) were measured. Electric field stimulated (EFS) contractions with and without atropine (cholinergic antagonist) were assessed. A separate group of bladders were denuded, homogenized and mitochondria quantified via citrate synthase activity. High-resolution respirometry measured oxygen consumption of individual electron transport chain complexes. Mitochondrial hydrogen peroxide (H2O2) emission was measured fluorimetrically using Amplex Ultra Red. Data were compared using a student t-test.

Results: HFD mice had a greater number of voids (p<0.005) and a larger void area (p<0.005). Blood glucose was greater in HFD (CD: 85 ± 6.1; HFD: 139 ± 5.9) as were body and overall visceral fat weights (p<0.0005). In contrast, HFD did not impact bladder weight. HFD did not change detrusor cholinergic contraction or adrenergic relaxation. However, detrusor contraction to EFS was markedly increased with HFD in both the presence and absence of atropine. Cholinergic inhibition blunted EFS contraction to a greater extent in CD bladders (CD: 75.7% ± 2.65; HFD: 42.3% ± 2.82, p<0.05) indicating that HFD had shifted EFS contraction to greater purinergic release. Citrate synthase activity indicated similar amounts of intact mitochondria. HFD resulted in a decreased respiratory capacity across 4 of 6 mitochondrial complexes investigated (p<0.05). Complex I and Complex II in state 4 conditions were unchanged. Additionally, mitochondrial H2O2 emission was increased in HFD bladders (CD: 11.4 pmols/min/mg ± 0.53; HFD: 18.6 ± 0.66, p<0.05).

Conclusions: Prolonged HFD leads to increased urinary frequency and volume, increased detrusor purinergic EFS contraction and mitochondrial dysfunction including decreased respiratory capacity and increased H2O2 emission. Further investigation of the role of mitochondrial dysfunction and resulting detrusor dysfunction following HFD is merited.
Title: Motor Unit Number Estimation of the External Anal Sphincter in Rats

Authors: C. Zhang¹ ², Y. Peng¹, Alvaro Munoz², Timothy Boone², Yingchun Zhang¹²*  

Affiliations: ¹ Department of Biomedical Engineering, University of Houston, Houston, TX, USA; ² Regenerative Medicine Program, Houston Methodist Research Institute, and Department of Urology, Houston Methodist Hospital, Houston, TX, USA

Introduction/Objectives: Reflexive and active control of the external anal sphincter (EAS) is essential for maintaining regulated colonic functions. Anorectal dysfunction, often related to compromised function of the EAS, is one of the major complaints in patients. Currently, the clinical diagnosis of anorectal dysfunction includes manometry, motor latency test, and single fiber electromyography (EMG). However, none of these techniques can be used to document the global innervation of the EAS in vivo. In this study, a novel approach was developed to non-invasively estimate the number of functioning motor units in the EAS. We further validated results with immunostaining of EAS in rats.

Methods: Compound muscle action potential (CMAP) responses were elicited by series of preset stimuli delivered intra-vaginally (Fig. 1a-c) to the pudendal nerve and the variation of the CMAPs was used to estimate the single motor unit potential (SMUP). The motor unit number estimation (MUNE) was implemented by calculating the ratio between the maximal CMAP and mean SMUP. Immunostaining of nicotinic acetylcholine receptors (AChRs) was used as a marker for identifying myofibers in the EAS. Linear regression analysis was performed to determine the relationship between MUNE and corresponding number of myofibers, with a significance level set at 0.05.

Results: The MUNE was successfully performed in 7 female rats, while immunofluorescence was implemented in 5 of them. The averaged SMUP was estimated as 45.00±17.24 μV, and the mean MUNE was calculated as 41±12 among the 7 rats tested. The results show good consistency with previous findings that the number of motoneurons innervating the EAS varies between 31.7 ± 8.5 to 73 ± 9.96 using retrograde tracing. The mean number of successfully identified myofibers was 580±45 myofiber/EAS, where a linear relationship between the MUNE and number of myofibers was observed (p<0.05).

Conclusions: This study represents the first effort to non-invasively assess the innervation of EAS in vivo using the rat as a pre-clinical model. The performance of the proposed MUNE approach was validated by comparison with immunostaining results, suggesting a consistent innervation ratio in rat EAS. This approach can potentially enable future clinical applications for advanced diagnosis and treatment of neurogenic EAS disorders.

Funding Sources: This work was supported by NIH DK082644, University of Houston, the Brown foundation, and Houston Methodist foundation

Figure 1 (a) System configuration (b) Linear fitting between NMJ Counting and MUNE
Title: A Preliminary Evaluation of Vaginal Alignment Following a Transvaginal Procedure Using MatriStem™ Pelvic Floor Matrix in the Rhesus Macaque

Authors: Deanna C. Easley¹*, William R. Barone¹, Pamela A. Moalli², Steven D. Abramowitch¹

Affiliations: ¹Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA ²Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA

Introduction/Objectives: Implantation of biological or synthetic mesh is the most common method of surgical intervention for Pelvic Organ Prolapse, however, complications ensuing from these surgical repairs occur in 15.5% of cases. Matristem™ (ACell, Inc.) Pelvic Floor Matrix is a urinary bladder matrix (UBM) device indicated for transvaginal repair. This device is remodeled and replaced by host tissue following implantation, which raises the concern that the process may result in a loss of support to the vagina. Thus, the goal of this study was to quantify measurable changes in vaginal alignment via magnetic resonance imaging (MRI) before (pre) and after (10 days and 3 months) a transvaginal procedure with this device in a rhesus macaque model.

Methods: Two rhesus macaques underwent a transvaginal procedure in accordance with the IACUC at the University of Pittsburgh (protocol #13081928). Level 1 & 2 support to the vagina was transected to simulate compromised support. Two sheets of 6-ply MatriStem™ were implanted to support the anterior and posterior vagina. Vaginal alignment was derived from MRIs taken pre, 10 days, and 3 months after surgery. The border of the vagina was manually traced, and used to calculate the centroid of each tracing. These centroids represent the path of the vagina through the pelvis. Further, a 3D coordinate system was mapped to the pelvis, and lines fit to the proximal and distal vagina were used to measure the angle of each line with respect to a cephalic oriented axis in the mid-sagittal plane, which is referred to as the angle of elevation.

Results: At 10 days, the angle of elevation became more acute by 8.6% and 17%, respectively. These changes reflect expectations of a tensioned transvaginal fixation of the vagina. At 3 months post surgery, angles of elevation approached pre surgery conditions, indicating that Matristem™ was providing a comparable level of support to native tissue, even following remodeling.

Conclusions: This preliminary study shows that Matristem™ repair appears to be re-establishing vaginal support. Future work will aim to increase sample size and compare data to non-repaired controls and those repaired using synthetic mesh.

Funding Source: ACell Inc. for funding these preliminary findings
Introduction/Objectives: Biomechanical simulation requires accurate representation of the geometry of the structures to be studied. Specifically, when simulating the interaction of implanted prosthetics with surrounding tissues, the geometric and mechanical properties of the prosthesis and the surrounding tissues need to be adequately represented. The present work describes methods for inserting test prostheses into MRI derived geometric models of the pelvic floor structures, in order to create realistic simulation models.

Methods: We modified an existing public domain image analysis software tool to allow placement and segmentation of arbitrarily shaped 3D objects into the output segmented geometry of a pelvic MRI image dataset. The tool was applied to create composite segmented geometry and 3D models of the MRI derived pelvic floor structures with the inserted prostheses in the intended anatomic locations, suitable for biomechanical simulation model creation.

Results: Segmentations of the organs in the source pelvic MRI datasets were created, showing the segmented embedded prostheses in the planned location. Three dimensional reconstructions of the segmented datasets were generated, which were viewable from multiple angles, and the ability to turn on and off all tissue and prosthesis layers was demonstrated.

Conclusions: We created a software application for inserting prostheses into segmented MRI based datasets. The output segmentations were suitable for input into a soft-tissue simulation tool suite, which generated simulation results suitable for analysis. This tool has the potential to enable patient specific, iterative surgical planning of prolapse repair strategies.

Funding Source: None
Title: Correlation of sacral nerve lead targeting and urological efficacy: motor mapping, electrode position, and stimulation amplitude

Authors: Lance Zirpel¹, Xin Su¹, Janine Wotton², Nicki Reinking³, Adam Steiner¹, Dwight E. Nelson¹, Thaddeus S. Brink¹

Affiliations: ¹Research & Core Technology Implantables, Medtronic, Minneapolis, MN USA, ² Dept. Of Psychology, Gustavus Adolphus University St. Peter, MN USA, ³ Physiological Research Laboratories, Medtronic, Minneapolis, MN USA

Introduction/Objectives: Sacral neuromodulation (SNM) is a clinically used therapy for refractory urge frequency and incontinent patients. Using a recently developed sheep model, this preclinical study retrospectively evaluated the relationship between implanted sacral lead locations, motor threshold values, motor mapping, and acute urological efficacy to determine if acute location and physiological measurements are correlated.

Methods: Twelve female polypay sheep were implanted with bilateral InterStim® devices (Model 3058) connected to quadripolar leads (model 3889) placed in the S2-4 foramina with S3 as the ideal target. CT scans at post-op and ≥12 months later were used for 3D rendering using MedCAD points placed on the sacrum and lead contacts to compare coordinates across animals. Acute cystometry was performed to test responses to SNM (0.21ms PW, 10Hz) at maximum tolerable amplitude (MTA). Motor threshold (MT) values were obtained by visual identification of the first motor response and the motor reflex was mapped to an anatomical map.

Results: Sheep were categorized as responders (n=6; 50%) or non-responders (n=6; 50%) based on ≥50% increase in bladder capacity to acute SNM. There was a significant difference in motor mapping areas between responders (peri-anal contractions) and non-responders (activation of leg) (Figure 1: chi-square; p<0.05). Higher MTA values correlated with larger bladder capacity increases (Pearson correlation; p<0.05). Contact position correlated with urological response (ANOVA; p<0.05). A generalized Procrustes analysis on the 17 leads in S3 (remainder in S2 or S4) showed variability of distributions was higher in distal contacts (0 & 1, mean distance to center 7.3 ± 1.8 mm, left & 6.8 ± 1.3 mm, right) than proximal (2 & 3, mean distance to center 5.8 ± 0.86 mm, left & 4.3 ± 0.35 mm, right (ANOVA; alpha = 0.05; F(3, 64) = 20.55; p< 0.0001).

Conclusions: 1. Responder sheep showed motor responses in peri-anal areas significantly more often than non-responders. 2. MTA weakly correlated with increased bladder capacity. 3. Activation of lead contacts proximal to the sacral foramen produced more reliable urological results than did activation of distal contacts. These results suggest well-positioned leads will elicit specific responses that could be essential to effective SNM therapy. Future work will characterize changes over time to provide a temporal correlation of this relationship.

Funding Source: Funding was provided by Medtronic.
Title: Metabolomics provides novel insights into the effect of diabetes on bladder detrusor and urothelial metabolism.

Authors: Yi Wang1, Gary G. Deng2 and Kelvin P. Davies1,3*

Affiliations: 1Albert Einstein College of Medicine, Department of Urology, Albert Einstein College of Medicine, Bronx, New York; 2Endocrine/Cardiovascular Research, Lilly Research Laboratories, Indianapolis, Indiana; 3Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, New York

Introduction/Objectives: Few studies have investigated the impact of diabetes, on the energy generating pathways of the bladder detrusor and urothelium. Such studies might identify strategies that can ameliorate diabetic bladder pathophysiology.

Methods: Streptozotocin was used to induce diabetes in 8 male Fischer rats for one month. Hyperglycemia was confirmed and urothelium and detrusor tissue isolated from both the diabetic and sixteen age matched non-diabetic bladder (controls). Metabolic profiling for each animal was performed by Metabolon Corp. ANOVA identified metabolites that differed significantly between groups and principal components analysis was used to reduce the dimension of the data.

Results: Comparison of the metabolome of control urothelium and detrusor revealed differential metabolism between the tissues, reflecting their different physiological functions. With diabetes there was an expected elevation in both the urothelium and detrusor of glucose, lactate, 2-3-hydroxybutyrate, branched-chain amino acid degradation products and primary, secondary bile acids and a reduction in 1,5-anhydroglucitol. In the diabetic detrusor there was an overall activation of the pentose-phosphate and polyol pathways, whilst oxidative phosphorylation and glycolysis was reduced. Interestingly, overall diabetes had fewer effects on the urothelium, except for significantly increased intermediates of the TCA cycle and lipid metabolism. In the control detrusor, analysis of the metabolome suggested a negative feedback mechanism regulating the glycolytic pathway which was unchanged with diabetes. In the urothelium there is no evidence of negative feedback in controls, but with diabetes there is a shift to negative feed-back of glycolysis. This suggests in the urothelium diabetes results in a change in expression or regulation of key enzymes involved in glycolysis. The metabolic profile provided evidence of oxidative stress after one month of diabetes; there was an elevation of the NADH/NAD ratio and compensatory increase in the GSH/GSSG ratio.

Conclusions: In addition to the expected effects of diabetes on bladder metabolism, there were striking differential changes caused by diabetes on the metabolism of detrusor and urothelium. The increased activity of the energy generating pathways in the detrusor would correlate with the reported over-activity of the diabetic bladder, whilst elevation of lysolipid and reduction of sphingolipid metabolism in the diabetic urothelium may alter membrane bioactivity (and thereby signaling mechanisms to the detrusor) as well as the barrier function of urothelium.

Funding Sources: Eli Lilly and Co. (LIFA fellowship); NIH/Diacomp 16GRU3659
Title: Temporal changes of detrusor muscle function in a high fat diet and low dose streptozotocin diabetic model: the compensated and decompensated states

Authors: Nicole Klee¹,², Robert S. Moreland²

Affiliations: Department of Physiology, Augusta University, Augusta, GA, USA¹ and Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia, PA, USA²

Introduction/Objectives: Bladder smooth muscle (detrusor muscle) undergoes temporal changes in response to diabetes resulting in diabetic bladder dysfunction (DBD). Type I DBD induces an early compensated state of bladder function exhibiting detrusor muscle remodeling and a hypercontractile phenotype progressing into a late decompensated state of bladder function and a hypocontractile phenotype. While the majority of DBD studies have been performed in type I diabetes, relatively few have been performed in type II diabetes, which accounts for ~90% of all diabetic cases. In a type II model, we aimed to establish a timeline of DBD. We hypothesized that force to exogenous stimulation would be

1. increased in the compensated state and positively correlated with myosin light chain (MLC) and phosphorylation calcium sensitization proteins (17 kDa protein-kinase C potentiated protein phosphatase 1 inhibitor (CPI-17) and myosin phosphatase targeting regulatory subunit (MYPT1))
2. decreased in the decompensated state and negatively correlated with MLC and phosphorylation calcium sensitization proteins (CPI-17 and MYPT1)

Methods: Type II diabetes was induced by utilizing a high fat diet (HFD; 45% fat), to induce insulin resistance and two low doses of streptozotocin (STZ; 30 mg/kg), to induce hyperglycemia and compromised pancreatic β-cell function. Functional studies were performed in urothelium denuded bladder strips in the presence of carbachol and ATP. Tissues were frozen at peak force in response to exogenous stimulation and used for western blot analysis of contractile proteins.

Results: The HFD/STZ model developed the compensated state at 1 week post-STZ and the decompensated state at 4 months post-STZ administration. Diabetic bladders were hypertrophied compared to control in both DBD states. The compensated state was characterized by increased volume per void and increased detrusor muscle contractility to exogenous addition of carbachol and ATP. The enhanced detrusor contractility to carbachol was not due to increased levels of myosin light chain (MLC) phosphorylation. The decompensated state was characterized by increased volume per void, number of voids, and contractility to ATP but not carbachol. This suggests that progression from the compensated to decompensated states involves decreased contractility to muscarinic stimulation. In addition, proteins involved with calcium sensitization and MLC demonstrated decreased phosphorylation levels.

Conclusions: In the HFD/STZ model, the compensated state was not mediated by changes in MLC phosphorylation. The decompensated state exhibited reduced force from 3 month post-STZ to 6 month post-STZ, which can be attributed to decreased phosphorylation of MLC and calcium sensitization proteins, CPI-17 and MYPT1.

Funding Sources: This project was funded by Diabetic Complications Program/ NIDDK and by NIH grant DK85734
Title: Sex differences in bladder dysfunction in response to enteric neuronal NFkB overactivation and experimentally induced colitis in mice.

Authors: Alan S. Braverman¹, Yonggang Zhang², Wenhui Hu², and Michael R. Ruggieri¹,³

Affiliations: ¹Department of Anatomy and Cell Biology, ²Department of Neuroscience, School of Medicine, and ³Electrical Engineering, College of Engineering, Temple University, and ³Shriners Hospitals for Children, Philadelphia, PA, USA

Introduction/Objectives: In experimental models, animals with induced colitis have increased urinary frequency and abnormalities in bladder contraction to cholinergic and electric field stimulation (EFS). Nuclear factor kappa B (NFkB) is a major mediator of the inflammatory response in inflammatory bowel disease and syndrome. The aim of the present study was to determine the effect of increased NFkB signalling in enteric neurons on bladder contraction with and without experimentally induced colitis.

Methods: Calretinin-Cre-ERT2-IKK2CA/tdTomato transgenic (TG) mice were generated and compared to littermate wild-type (WT) controls. At two months of age, the mice were treated for three days with tamoxifen causing continuous NFkB activation in enteric neuronal cells. One month later, the mice were treated with vehicle or 2.5% dextran sulfate sodium (DSS) for 7 days to induce colitis. Full thickness urinary bladder strips were stimulated with potassium and then EFS to induce nerve mediated contraction. The effect of atropine to inhibit muscarinic receptors and alpha, beta methylene ATP (ATP) to desensitize purinergic receptors was determined.

Results: Even though the bladder contractile response to high potassium stimulation was similar between sexes in both WT and TG mice, the EFS induced maximal contraction was greater in WT males than in WT females. Bladder strips from WT female mice had a significantly greater atropine resistant (purinergic) component of EFS induced contraction, compared to WT male mice, but this was not found in TG mice. The cholinergic component of EFS induced contraction was not different between male and female mice (WT or TG).

Acute colitis significantly reduced KCl stimulated and nerve mediated contraction in bladder strips from WT female and male mice, and from TG female mice. Constitutive activation of neuronal NFkB preserved the nerve mediated and KCl induced contractile response in male mice. Acute colitis increased the purinergic component of nerve mediated contractions in male WT mice only.

Conclusions: Experimentally induced colitis causes decreased bladder contraction. Neuronal NFkB signalling rescued the decreased bladder contraction due to colitis, perhaps through purinergic regulation in males. Neurogenic inflammation in the bladder may cause a larger degree of bladder dysfunction in female than in male mice perhaps because females have a greater purinergic component of nerve mediated bladder contraction that does not further increase due to colitis.

Funding Sources: RO1DK075684 and P20DK097819
Title: Functional Relevance of Purinergic P2X4R in Bladder Smooth Muscle.

Authors: Vivian Cristofaro1,3*, Josephine A. Carew2,3, Sean D. Carey1,3, Raj K. Goyal2,3 and Maryrose P. Sullivan1,3

Affiliations: 1Division of Urology and 2Department of Medicine VA Boston Healthcare System, 3Harvard Medical School; Boston MA, USA.

Introduction/Objectives: Under physiologic conditions in animals and pathologic conditions in humans, purinergic mechanisms contribute significantly to detrusor contractions. Although the activation of P2X1 receptors (P2X1R) accounts for the largest portion of bladder smooth muscle (BSM) responses to ATP, previous reports have shown that P2X1R antagonists do not completely abolish the purinergic component of neurogenic contractions, suggesting the presence of other P2XR subtypes on BSM. P2X4R has been identified in bladder tissue, however whether this receptor is functionally relevant warrants investigation. The aim of this study was to examine the extent of P2X4R expression in BSM tissue and to investigate its pharmacological contribution to ATP-mediated detrusor contractions.

Methods: P2X4R mRNA and protein expression was investigated in mouse bladder tissue (without mucosa) and in cultured BSM cells by real-time rt-PCR and western blotting respectively. In vitro isometric tension studies were performed in mouse BSM strips without mucosa. Purinergic detrusor contractions were elicited by administration of α-β-methylene-ATP (αβmATP), and the purinergic component of neurogenic contractions induced by electrical field stimulations (EFS) was isolated by pre-treatment with the muscarinic receptor antagonist atropine. The inhibitory effect of two P2X4R selective antagonists, 5-BDBD and BX430, on the αβmATP- and EFS-induced contractions was investigated in the presence of P2X1R antagonist NF449. In addition, the effect of the P2X4R positive modulator ivermectin (IVC) on αβmATP responses was investigated. Data were analyzed by a repeated measures analysis of variance.

Results: P2X4R mRNA was detected in smooth muscle from both bladder tissue and cultured BSM cells, although its expression was significantly lower than P2X1R expression. Immunoreactivity for P2X4R was detected in lysates from both mouse BSM tissue and smooth muscle cells. Functional studies indicated that although P2X1R activation is predominantly responsible for purinergic contractions in mouse detrusor, a significant portion of the contractile response to both αβmATP (22.3±7%) and EFS (27.5±4% of purinergic component of EFS) was resistant to P2X1R inhibition. This NF449-resistant component was abolished by administration of P2X4R antagonists 5-BDBD or BX430. In addition, responses to αβmATP increased significantly upon administration of IVC.

Conclusions: The expression of P2X4R in detrusor smooth muscle together with the identification of a P2X4R-sensitive component of bladder contractions suggest that the activation of this P2X receptor subtype could significantly contribute to ATP-mediated BSM responses. P2X4R may thus potentially represent a novel target for the management of detrusor dysfunctions associated with alterations in purinergic signaling.

Funding Source: Department of Veterans Affairs, Research Service BX001790; BX002806.
Title: Aberrant bladder reflexes can drive hind limb locomotor activity following complete suprasacral spinal cord injury

Authors: Brian M. Inouye1, Jillene M. Brooks2, Danielle J. Degoski2, Francis M. Hughes Jr1, J. Todd Purves1, Matthew O. Fraser1,2,3

Affiliations: 1Division of Urology, Duke University Medical Center, Durham, NC; 2Institute for Medical Research, Durham, NC; 3Durham Veterans Affairs Medical Center, Durham, NC

Introduction/Objectives: Many rats with chronic suprasacral spinal cord injury (SCI) demonstrate hind limb locomotor activity (HLLA) in response to external crede or high pressure contractions during cystometry. We propose that this aberrant, pressure-driven bladder reflex pathway may be harnessed to facilitate walking in SCI patients. As a first step in exploring this possibility, we examined the relationship between intravesical pressure (IVP) and HLLA in chronic suprasacral SCI rats.

Methods: Female rats (4 weeks post-SCI at T9-10, n=16) were anesthetized with isoflurane and fitted with transvesical catheters and right quadriceps EMG electrodes to monitor bladder and hind limb locomotor activities, respectively. The animals were mounted in Ballman restraint cages to which they had been previously acclimated. The catheter was connected to a pressure transducer, an infusion pump, and a saline-filled reservoir mounted on a metered vertical pole (pressure clamp). After 30 min of recovery from anesthesia, the bladder was filled at 0.1 ml/min with saline to verify bladder-to-bladder reflex activity for 30 min. IVP was then increased in an interrupted stepwise fashion from 0-120 cmH2O at 10 cmH2O increments. Each step consisted of five minutes: 3 minutes at the new pressure followed by 2 minutes at 0 cmH2O. IVP and the number of HLLA events (as defined by rhythmic EMG discharges of 3-10 cycles/event) were recorded for each pressure step. This process was repeated for two more trials for each rat to assess the durability of the reflex. Data were analyzed using ANOVA with repeated measures both within and across pressure escalation trials. P<0.05 was considered significant.

Results: ANOVA revealed that locomotor events increased with increasing intravesical pressure and decreased with the number of escalation trials (P<0.0001 for both effects). The increase in the number of locomotor events with increasing intravesical pressure appeared to plateau at ~50-60 cmH2O (P<0.05 for all). The average of the maximal number of locomotor events for each animal decreased steadily from ~3.0, 2.5 and 1.75 over the three trials.

Conclusions: There is a positive relationship between IVP and HLLA that suggests the emergence of an aberrant bladder-to-hind limb locomotor reflex pathway following SCI. It may be possible to harness this reflex pathway independently of the state of the bladder to facilitate walking in SCI patients.

Funding Source: VA RRD SPIRE RX-001749-01
Title: Spinal Cord Injury and Detrusor PDGFRα⁺ Cells

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

Affiliations: Department of Physiology & Cell Biology, University of Nevada Reno, School of Medicine, Reno, NV, USA

Introduction/Objectives: Neurogenic bladder dysfunction due to spinal cord injury (SCI) poses a significant threat to the well-being of patients. The complications of this condition include but are not limited to incontinence, renal impairment, urinary tract infection, stones, and poor quality of life. Clinical manifestations of SCI involve combination of storage and voiding bladder problems. Although a number of clinical studies have reported overactive bladder (OAB) after SCI, the pathophysiological mechanisms remain unclear. SCI is widely used to induce neurogenic bladder in rodent models. These animals exhibited dysfunctional condition results in different symptoms, ranging from acute urinary retention to an overactive bladder or a combination of both. There is an abundance of PDGFRα⁺ cells in detrusor muscles. This cell involves the membrane stabilization via activation of SK channels in detrusor PDGFRα⁺ cells during filling. Thus we investigate the molecular and protein expression of PDGFRα⁺ cells from SCI mice to characterize the role of these cells that contributes to development of OAB in SCI.

Methods: SCI was induced by complete compression of T12-L1 spinal cord. Experiments were performed on 24 hr, 48 hr and 72 hr after surgery. We employed molecular approaches and ex vivo cystometry. Pdgfra and Kcnn1–3 transcripts were analyzed for molecular expression. Ex vivo compliance was used for testing SK channel sensitivity in control and SCI mice.

Results: In quantitative analysis of transcripts, Pdgfra and Kcnn3 transcripts in SCI detrusor were significantly decreased in a time-dependent manner after SCI surgery compared with control detrusor. In ex vivo cystometry, SCI bladder revealed an increase in the amplitude and frequency of non-voiding pressure responses during filling. Effects of a SK blocker (apamin) and a SK channel activator (SKA-31) were reduced in non-voiding contractions in SCI mice compared to control.

Conclusions: These findings support that downregulation of PDGFRα⁺ cells and SK channels in SCI detrusors might involve the development of OAB in SCI.

Funding Source: Supported by NIDDK, RO1 DK098388
Title: NLRP3/IL-1β mediates Denervation During Bladder Outlet Obstruction in Rats

Authors: Robin Lutolf¹,², Francis M. Hughes, Jr.², J Todd Purves²

Affiliations: ¹Department of Health Science and Technology, ETH Zurich, Switzerland, ²Division of Surgery, Department of Urology, Duke University Medical Center, Durham NC, US

Introduction/Objectives: Bladder outlet obstruction (BOO) is a common condition resulting from benign prostatic hyperplasia, neurologic pathology, organ prolapse, etc. Long-term, obstruction is well-established to evoke denervation in the bladder which causes the detrusor to become hypocontractile, resulting in inefficient bladder emptying and consequent infections, continence issues or even renal failure. Recently, considerable attention has been paid to a role for inflammation in bladder deterioration during BOO and we have shown a central role for the NLRP3 inflammasome in triggering this inflammation. In the present study we explore a possible connection between this NLRP3-induced inflammation and bladder denervation.

Methods: Rats were divided into 5 groups: 1) control, 2) sham operated, 3) BOO+Vehicle (1ml, 40% ethanol in PBS, p.o.), 4) BOO+Glyburide (Gly, NLRP3 inhibitor; 10 mg/kg, p.o.), 5) BOO+Anakinra (Ana, IL-1 receptor antagonist; 25 mg/kg, i.p.). BOO is constructed in female rats by inserting a 1 mm outer diameter transurethral catheter, tying a silk ligature around the urethra and removing the catheter. Medications were administered prior to surgery and once daily. At 12 days animals were sacrificed and the bladders processed for histological analysis. Transverse sections (5 µm) were stained for PGP9.5 expression (a pan-neuronal marker) using standard immunohistochemistry techniques. Entire sections were scanned, using a 10X objective, into TIFF files using Zen software (Zeiss inc.). Images were imported into Elements software (Nikon inc.) and the area of individual neurons designated as well as total bladder area (exclusive of the urothelia and lumen). The number of neurons and respective areas were used to calculate nerve density.

Results: Denervation in the bladder wall during BOO was significant, as measured by nerve density. This effect was attenuated by either preventing NLRP3 activation with Gly or blocking IL-1β’s action at its receptor by treatment with Ana, clearly indicating a role for NLRP3/IL-1β in bladder denervation during BOO. The effect was also apparent with the total number of nerves despite considerable changes in bladder wall area (increased in BOO, maintained by Gly or Ana). Interestingly, the mean area of individual nerves was increased in BOO. This effect was blocked by Gly or Ana suggesting a loss of smaller neurons and/or retraction of neuronal branching during BOO as a result of NLRP3/IL-1β.

Conclusions: NLRP3/IL-1b-induced inflammation leads to bladder denervation during BOO and blocking this pathway, either by preventing NLRP3 activation or inhibiting the action of IL-1B, prevents nerve loss.

Funding Source: NIDDK: R01DK103534 (PI - Purves)
Title: Micturition and defecation behavior following pelvic decentralization in a canine model.

Authors: Michael R. Ruggieri, Sr.¹,²,³, Ekta Tiwari², Alan S. Braverman¹, Mary F. Barbe¹.

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

Introduction/Objectives: We demonstrated motor reinnervation of the decentralized canine urinary bladder by increased detrusor pressure induced by electrical stimulation of the transferred genitofemoral or femoral nerves in 21 of 28 animals.¹ We previously reported evidence of bladder and bowel fullness sensation in these new neuronal pathways based on observation of micturition and defecation postures in the reinnervated animals.² Our focus here was to determine whether these postures are eliminated in decentralized animals.

Methods: The pelvis was decentralized by bilateral transection of the hypogastric nerves and all spinal nerve roots caudal to L7 in 6 female canines. A separate group of 6 female canines was decentralized identically with the additional bilateral transection of L7 dorsal roots. Video surveillance of the housing cages allowed measurement of the frequency and duration of urination and defecation postures at monthly intervals post operatively (PO) by observers blinded to the surgical interventions.

Results: In the 6 animals with intact L7 dorsal roots, 3 displayed micturition postures 1 month, 6/6 at 2 and 3 months and 5/6 at 4 months PO. In the 6 animals with the additional L7 dorsal root transections, micturition postures were observed in 2/6 at 1 and 2 month and 3/6 at 3 and 4 months PO. In the L7 dorsal root intact animals, defecation postures were observed in 0/6 at 1 month and 2/6 at 2, 3 and 4 months PO. In the L7 dorsal root transected group, 1/6 showed defecation postures at 1 and 2 months but not at 4 months, and 1/6 showed these postures at 3 and 4 months PO.

Conclusions: In the L7 dorsal root intact animals, although half were unable to sense bladder fullness and 0/6 could not sense bowel fullness at 1 month PO, all were able to sense bladder fullness at 2 and 3 months and all but 1 at 4 months PO, suggesting sprouting of sensory bladder innervation likely from the remaining lower lumbar dorsal root fibers. Fewer dogs with L7 dorsal root transected were able to detect bladder fullness. The ability of 3/6 animals in the L7 dorsal root transected group to sense bladder fullness at 2-4 months PO may be from sensory nerve sprouting or variations in the bladder sensory innervation, as we described previously.³ Bowel fullness sensation appears to be primarily mediated by sacral sensory innervation.

Funding Source: NIH 1R01NS070267

Literature Cited:
Title: Sonic hedgehog promotes sprouting of neurons in the pelvic ganglia and cavernous nerve during regeneration

Authors: Ryan Dobbs¹, Shawn Choe¹, Daniel A. Harrington², Samuel I. Stupp³, Kevin T. McVary⁴, Carol A. Podlasek⁵*

Affiliations: ¹Department of Urology, University of Illinois at Chicago, Chicago, IL 60612, ²Department of Biosciences, Rice University, Houston, TX 77005, ³Simpson-Querrey Institute for BioNanotechnology, Department of Chemistry, Department of Materials Science and Engineering, and Biomedical Engineering, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, ⁴Division of Urology, Southern Illinois University School of Medicine, Springfield, IL 62794, ⁵Departments of Urology, Physiology and Bioengineering, University of Illinois at Chicago, Chicago, IL 60612

Introduction/Objectives: We’ve shown in previous studies that sonic hedgehog (SHH) protein delivered by nanoparticle based peptide amphiphile (PA) hydrogels to the cavernous nerve at the time of crush injury (mimicking prostatectomy), are neuroprotective and promote cavernous nerve (CN) regeneration in a rat model. The mechanism of how SHH promotes CN regeneration is unknown. We hypothesize that SHH promotes sprouting of CN axons, in order to enhance nerve regeneration. We examine this hypothesis in an in vitro organ culture model.

Methods: The caudal portion of the pelvic ganglia (innervates penis) and cavernous nerve were dissected from adult Sprague Dawley rats (n=47) and placed in Matrigel in growth factor reduced medium and were grown for three to five days. Pelvic ganglia 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 PBS/mouse serum albumin (MSA) protein. Sprouting was evaluated for number of sprouts and their length, and by immunohistochemical analysis for sprouting markers (GAP43 and nNOS).

Results: Sprouting of pelvic ganglia and cavernous nerve axons was increased with SHH treatment. Sprouts were more abundant, longer in length, with larger arborization of sprouts, in comparison to controls. More sprouting was promoted with SHH treatment of CN injured nerves. The CN had similar sprouting potential at 4 and 9 days after crush injury. Localization of SHH delivery makes a difference in sprouting potential.

Conclusions: The mechanism of how SHH PA treatment promotes CN regeneration, involves enhanced sprouting of pelvic ganglia and CN neurons. Understanding the mechanism of SHH PA action on neuronal tissue is critical for translation to prostatectomy patients and to further enhance regeneration.

Funding Source: NIH/NIDDK DK079184
Title: Determining integrity of the nerve-smooth muscle functional unit of the bladder after long-term decentralization

Authors: Danielle M. Salvadeo¹, Mary F. Barbe ¹, Nagat Frara¹, Ekta Tiwari¹, Alan S. Braverman¹, Michael R. Ruggieri, Sr. ¹,²

Affiliations: ¹Department of Anatomy and Cell Biology, Lewis Katz School of Medicine at Temple University, 3500 North Broad Street, Philadelphia, PA 19140; ²Shriners Hospitals for Children of Philadelphia, 3551 North Broad Street, Philadelphia, PA 19140

Introduction/Objectives: Somatic nerve transection causes rapid loss of skeletal muscle mass and contractility. While skeletal muscle degeneration following nerve injury has been well investigated, less is known about the effects of autonomic nerve transection on smooth muscle. We explored changes in the nerve-smooth muscle functional unit following sacral root decentralization to determine integrity after decreased innervation.

Methods: Female mixed-breed hound dogs were surgically decentralized by bilateral transection of all spinal roots caudal to L7, including the dorsal root of L7 in a subgroup. Three weeks prior to the terminal surgery, bladders were injected cystoscopically with Fluorogold around the ureterovesical junction for retrograde neuronal labeling. Tissue function was tested during the terminal procedure after 6-month (n=2) and 12-month (n=6) decentralization and compared to sham/unoperated control animals (n=13). Immediately prior to euthanasia, in vivo detrusor pressure after stimulation of nerves originating from the pelvic plexus (e.g. the anterior vesicle branch) were recorded. Collected bladder and pelvic plexus tissues from controls and 6-month decentralized dogs were harvested (n=3-6/group), cryosectioned, and examined for Fluorogold labeling. Bladder tissues were stained for caspase-3 and immunostaining was quantified. Gastric tissue and red blood cells within bladder walls were used as positive controls. Data was analyzed using unpaired ANOVA.

Results: Nerve stimulation caused a robust increase in detrusor pressure in both control and decentralized groups. Likewise, abundant Fluorogold-labeled neuronal cell bodies were observed in ganglia in the pelvic plexus of both sham and decentralized animals. Immunohistochemical stain for caspase-3 showed no difference across groups. Also, we did not observe co-localization of Fluorogold-positive neuronal tissue and caspase-3, or presence of caspase-3 in smooth muscle fibers of the bladder wall.

Conclusions: The presence of Fluorogold-labeled pelvic plexus ganglia in decentralized animals demonstrates that the ganglia remained intact up to 6 months after decentralization. Caspase-3 staining results showed no increase in apoptosis in the neuronal tissues or bladder smooth muscle in decentralized dogs, suggesting no increased apoptotic cell death. No significant difference between detrusor pressure responses across groups after nerve-evoked stimulation indicates that the nerve-smooth muscle functional unit of the bladder is intact up to 12 months after injury and therefore, nerve reinnervation strategies could be successful.

Funding Source: NIH-NINDS NS070267
Title: Sphingosine-1-phosphate in vitro and in vivo modulates corpus cavernosum smooth muscle tone

Authors: Xinhua Zhang¹,², Jing Yin¹,², Nirmala D Kanika², Yuehong Tong², Guillermo Villegas², Arnold Melman² and Michael E DiSanto¹,²

Affiliations: ¹ Department of Biomedical Sciences and Surgery, Cooper Medical School of Rowan University, Camden, NJ, USA, ² Department of Urology, Albert Einstein College of Medicine, Bronx, NY, USA

Introduction/Objectives: The bioactive lipid sphingosine-1-phosphate (S1P) regulates smooth muscle (SM) contractility predominantly via three G protein-coupled receptors. The S1P1 receptor is associated with nitric oxide-mediated SM relaxation while S1P2 & S1P3 receptors are linked to SM contraction via activation of the Rho-kinase (ROK) pathway. The objective of this study was to determine the role of S1P in the modulation of corpus cavernosum (CC) SM (CCSM) tone.

Methods: Human and rat samples were used. Plasma S1P levels were detected by high-performance liquid chromatography. The expression of S1P1-3 receptors and sphingosine kinase-1 (SphK1) was determined by Real-Time RT-PCR and Western Blot. In vitro organ bath contractility and in vivo intracavernous pressure (ICP) measurements were performed. Results were expressed as mean ± SEM for n experiments. Statistical analysis was performed using either the Student's t-test (when two sample treatments were being compared) or using ANOVA when multiple means were compared. p<0.05 was considered significant.

Results: Plasma S1P levels were determined to be ~200 nanomolar. Human and rat CC both express SphK1 and all S1P1-3 receptors. Exogenous S1P and the S1P receptor agonist FTY720-P contracted while antagonist JTE-013 relaxed CCSM in vitro. Meanwhile, force produced by S1P and agonists could be totally reversed by ROK inhibitor. Also, intracavernous injection of FTY720-P inhibited ICP rise induced by submaximal electrical stimulation of cavernous nerve, while JTE-013 alone induced ICP increase in vivo. Finally, SphK1 siRNA knocked down rat CC SphK1 by 80% and ICP were significantly potentiated.

Conclusions: In conclusion, we provide novel data that S1P, possibly coupling S1P2 and S1P3 receptors via RhoA/ROK pathway, mediates CCSM in vitro and in vivo. Antagonizing S1P or its receptors induces CCSM relaxation and proerectile effects. Thus, we provide the first clear evidences that the S1P system is another key contractile regulatory system in CC and thus this pathway is a potential therapeutic target for the treatment of priapism and erectile dysfunction.

Funding Source: NIH R01 DK077116 to MD.
Title: Neurotrophin Dysregulation after Dual Injury Childbirth Model is corrected via Electrical stimulation of the Pudendal Nerve in a Rat Model

Authors: Brian M. Balog\textsuperscript{1,2,3}, Dan Li Lin\textsuperscript{2}, Brett Hanzlicek\textsuperscript{2}, Margot S. Damaser\textsuperscript{1,2}

Affiliations: Department of Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio, USA\textsuperscript{1}, Advanced Platform Technology Rehabilitation R&D Center of Excellence, Louis Stokes Cleveland Veterans Medical Center, Cleveland, Ohio, USA\textsuperscript{3}, Department of Biology, University of Akron, Akron, Ohio, USA\textsuperscript{3}.

Introduction/Objectives: Stress Urinary Incontinence (SUI), the leakage of urine due to an increase in abdominal pressure, affects 30\% of women over the age of 40. Childbirth is the primary risk factor for SUI since the child’s head passing through the birth canal can injure the EUS and the nerve that innervates it, the pudendal nerve (PN). Animal models with combined EUS (Vaginal distension (VD)) and PN crush (PNC) injuries have shown that the nerve does not recover as well as with PNC alone and the animals have delayed return of function. Typically after a nerve injury, the neuron upregulates neurotrophin factors (i.e. Brain Derived Neurotrophic factor (BDNF)) and their receptors (i.e. Tyrosine kinase B (Trk B) and p75), Schwann cells also upregulate neurotrophin factors (i.e. Glial derived Neurotrophin factor (GDNF) and NT4) as part of the regeneration process. The impaired nerve regeneration in the dual injury model was shown to be due to a dysregulation of BDNF in the PN motoneuron, so any potential treatment would need to upregulate BDNF. Electrical Stimulation (ES) has been shown to upregulate BDNF and Trk B in neurons and GDNF in Schwann cells. Our previous work has shown daily and 4 times per week stimulation for 2 weeks accelerates functional recovery after PNC+VD. The aim of the study was to determine if daily stimulation causes an upregulation of neurotrophin factor and their receptors in motoneurons of the PN after injury. We hypothesize that the accelerated recovery from ES is mediated by an upregulation of BDNF and NT4 and their receptor Trk B, as well as GFRα1, the receptor of GDNF.

Methods: Animals were divided into 5 groups: Sham PNC, PNC, PNC+VD, PNC+VD with sham stimulation (SS) or PNC+VD+ES. ES (20 Hz, 0.3mA, 0.1ms) was conducted daily for 1 or 2 weeks after injury, when the spinal cord was harvested from L4-S3. The spinal cord was sectioned and Onuf’s nucleus, the motoneuron nucleus for the PN, was harvested via laser capture microscopy. RNA was then isolated and preamplified before running PCR and compared to GAPDH expression. A one way Anova was used to determine statistically significant differences between groups.

Results: One week after PNC+VD, BDNF was not upregulated as previously shown. Neither were p75 or GFRα1, as expected. NT4 was not downregulated after PNC+VD, as expected. P75 was significantly increased in the PNC+VD+ES group compared to the PNC+VD group 2 weeks after injury. BDNF was also upregulated 2 weeks after PNC+VD+ES compared to PNC+VD.

Conclusions: P75, NT4 and GFRα1 expression data suggest that PNC+VD causes dysregulation of other regenerative proteins other than BDNF. BDNF was upregulated 2 weeks after PNC+VD with ES. Future research will be designed to investigate the mechanism of this effect.

Funding Sources: VA Merit A1262-R and the RR&D Service of the VA
Title: Controlled release of IGF1 enhances urethral sphincter function and histological structure in the treatment of female stress urinary incontinence in a rodent model

Authors: Liren Zhong¹, Hao Yan², Yaodong Jiang¹, Jian Yang¹, Emmanuel Opara¹, Margot S. Damaser²,³, and Yuanyuan Zhang¹

Affiliations: ¹ Institute for Regenerative Medicine, Wake Forest University, Winston-Salem, NC, USA, ² Glickman Urological and Kidney Institute and Lerner Research Institute of the Cleveland Clinic, Cleveland, OH, USA, ³ The Advanced Platform Technology Center of the Louis Stokes Cleveland VA Medical Center, Cleveland, OH

Introduction/Objectives: IGF1 plays a key modulatory role in promoting angiogenesis, as well as muscle and nerve regeneration. Although systemic administration of IGF1 improved urinary sphincter function in vivo, this therapy has several pitfalls, including limited effects due to the short half-life of IGF1 and potential dose-related toxicity. It is important to use a delivery system that slowly release suitable amounts of IGF1 over time. The goal of this study was to determine the effects of controlled release of IGF1 from alginate-gelatin microbeads (IGF1-A-G-beads) on sphincter tissue repair in a rat model of stress urinary incontinence (SUI).

Methods: Forty-four female SD rats were randomized into 4 groups (G) and underwent vaginal distension (VD) followed by periurethral injection of IGF1-A-G-beads (1x10⁴ beads/1 ml normal saline) (G1), VD + empty microbeads (G2), VD + normal saline (NS), or sham VD + NS. Urethral function (leak point pressure, LPP) and histology were assessed one week after VD injury. Quantitative data was analyzed using ANOVA on Ranks followed by a Tukey posthoc test with p < 0.05 indicating a statistically significant difference between groups. Data is presented as mean +/- standard error of the mean.

Results: LPP was significantly decreased with VD when treated with saline (23.9 ± 1.3 cmH2O) or with empty microbeads (21.7 ± 0.8 cmH2O) demonstrating that the microbeads themselves do not create a bulking or obstructive effect in the urethra. LPP significantly increased in rats with VD treated with IGF1-A-G-beads (28.4 ± 1.2 cmH2O), compared to those treated with empty microbeads and was not significantly different from LPP of rats with sham VD (44.4 ± 3.4 cmH2O), demonstrating initiation of a reparative effect even 1 week after VD. Histological analysis demonstrated well-developed, well-organized skeletal muscle fibers in the external urethral sphincter of rats with VD treated with IGF1-A-G-beads, similar to that of sham-injured animals. In contrast, substantial muscle fiber attenuation and disorganization was observed in VD injured, NS- or empty microbeads- treated rats.


Funding Source: NIH NIDDK R56 DK100669-01A1
Title: Aging Effects on the Central Motor Control of the External Anal Sphincter in Women

Authors: Yun Peng¹, Jinbao He², Rose Khavari³, Timothy B. Boone³, Yingchun Zhang¹,*

Affiliations: ¹ Department of Biomedical Engineering, University of Houston, Houston, TX, USA; ² Ningbo University of Technology, Ningbo, Zhejiang, China; ³ Regenerative Medicine Program, Houston Methodist Research Institute, and Department of Urology, Houston Methodist Hospital, Houston, TX, USA

Introduction/Objectives: Accumulated clinical evidence has shown that aging is associated with deficits in the central nerve system (CNS) that leads to disruption in descending excitation. Whether this is true in pelvic floor muscles remains unclear. This study aimed to study whether there is evidence of an aging-associated decreasing descending excitation to the motoneuron pool of the external anal sphincter (EAS) in women.

Methods: Surface electromyography (EMG) signals of the EAS were acquired from 14 female subjects (38.6±16.6 years, range 20-73 years, no anorectal disorders) during maximum squeeze using a high-density rectal probe (Fig 1a). Motor unit (MU) action potentials were separated using our K-means clustering and convolution kernel compensation algorithm, with the firing rate of each MU obtained. The mean MU firing rate, a key marker to assess the motor control of the CNS, was calculated for all decomposed MUs for each subject. Linear regression analysis was performed to study the impact of aging on the mean MU firing rate.

Results: The mean MU firing rate was 7.4±2.1Hz. Linear regression showed a decreasing, though not significant, trend with advancing age (p=0.10) (Fig 1b), suggesting a possible deficit in the CNS of aged women in driving the lower motoneurons to activate the EAS. Our ongoing recruitment of more aged women will help us consolidate this observation, to offer valuable insights into the mechanism of aging-associated anorectal disorders.

Conclusions: This study represents the first effort to use advanced high-density surface EMG analysis to noninvasively evaluate aging effects on the central motor control of the EAS in women.

Funding Sources: This study was supported by NIH DK082644 and the University of Houston.

Figure 1 (a) High-density intra-rectal surface EMG probe and (b) Linear fitting between mean MU firing rate and age.
Title: Systemic Treatment of Stress Urinary Incontinence with Human Urine-Derived Stem Cells

Authors: Christine Tran,1 Abhi Tangada,1 Hualin Yi,2 Brian Balog,1,3 Yuanyuan Zhang,2 and Margot S. Damaser1,3*

Affiliations: 1Glickman Urological and Kidney Institute and Lerner Research Institute of the Cleveland Clinic, Cleveland, OH, USA; 2Institute for Regenerative Medicine of the Wake Forest School of Medicine, Winston-Salem, NC, USA; and 3the Advanced Platform Technology Center of the Louis Stokes Cleveland VA Medical Center, Cleveland, OH

Introduction/Objectives: Stem cell based therapy has emerged as a promising treatment alternative for stress urinary incontinence (SUI). Human urine-derived stem cells (USCs) have no risk from biopsy and therefore may be a better option than other adult stem cells. Possible therapeutic mechanisms of action of stem cells are homing of cells to areas in need of repair and secretion of bioactive paracrine factors acting systemically as well as locally. We hypothesized that USCs or their secretome alone would promote functional recovery in a rodent model of female SUI even when given systemically.

Methods: Thirty two-female Sprague-Dawley rats were randomized into 3 groups and underwent vaginal distension (VD) followed by intraperitoneal (ip) delivery of USCs (VD + USCs) VD and ip delivery of saline as a vehicle control, or sham VD with ip saline. Three additional groups (32 rats) were utilized to investigate if factors secreted by USCs alone facilitate recovery from VD: concentrated conditioned media (CCM) from USCs given ip after VD, concentrated control media (CM) given ip after VD, and CM given ip after sham VD. All treatments were given 1 hour after VD or sham VD. CCM was generated by incubating confluent USCs in serum-free media for 24 hours. Cultured supernatant was then extracted, washed, and concentrated to form CCM. One week after injury, treatment efficacy was assessed by measurement of leak point pressure (LPP), and qualitative anatomical assessment of the urethra. Quantitative data were analyzed by one-way analysis of variance (ANOVA) and Holm-Sidak posthoc tests with p<0.05 indicating a significant difference.

Results: LPP significantly increased after VD in rats treated with USCs or CCM, compared to animals that received saline, but not significantly different from sham VD. Collagen infiltration of striated muscle in the external urethral sphincter, along with substantial muscle fiber attenuation and disruption of the striated muscle layers were noted, as has been observed previously in this model. External urethral sphincter structure was greatly improved with USC implantation, and was more similar to that of sham-injured animals than injured rats treated with saline. Elastin fibers in VD + USCs and VD + CCM animals were long, thickened, and mostly oriented compared to the short, thin, and disoriented fibers in VD + saline and VD + CM animals. No human USCs were found in the region of urethra.

Conclusions: Ip injection of USCs and their secretions facilitate recovery from SUI in a rat model, likely via systemic and paracrine mechanisms. Elastogenesis may play a role in recovery of urethral function. USCs represent an attractive, alternate stem cell source with no biopsy risk to target the underlying pathophysiology in SUI.

Funding Sources: NIH NIDDK R56 DK100669-01A1, the Research Projects Committee of the Cleveland Clinic, and the Rehabilitation R&D Service of the Department of Veterans Affairs.
Title: Optimization of Sonic hedgehog delivery from self-assembled nanofiber hydrogels

Authors: Shawn Choe¹, Daniel A. Harrington², Samuel I. Stupp³, Kevin T. McVary⁴, Carol A. Podlasek⁵*

Affiliations: ¹Department of Urology, University of Illinois at Chicago, Chicago, IL 60612, ²Department of Biosciences, Rice University, Houston, TX 77005, ³Simpson-Querrey Institute for BioNanotechnology, Department of Chemistry, Department of Materials Science and Engineering, and Biomedical Engineering, Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, ⁴Division of Urology, Southern Illinois University School of Medicine, Springfield, IL 62794, ⁵Departments of Urology, Physiology and Bioengineering, University of Illinois at Chicago, Chicago, IL 60612

Introduction/Objectives: Sonic hedgehog (SHH) protein delivered by nanoparticle based peptide amphiphile (PA) hydrogels to the penis suppress apoptosis in a rat cavernous nerve (CN) resection model. We examine the hypothesis that SHH PA will suppress morphology changes in the penis in a CN crush model that more readily reflects injury observed in prostatectomy patients. Optimization of delivery conditions is essential for clinical translation.

Methods: Bilateral CN crush was performed on Sprague Dawley rats (n=67) and SHH or mouse serum albumin (MSA, control) protein was delivered by PA injected into the corpora cavernosa. Rats were sacrificed after 4 and 9 days. 2X SHH protein was also assayed at 4 days. A second SHH PA injection at 5 days occurred prior to sacrifice at 9 days. Additional rats had SHH or MSA delivered to both the penis and CN by PA. TUNEL and hydroxyproline assay were performed.

Results: Apoptosis increased 54% 4 days after injury (p=0.0001). SHH PA suppressed apoptosis 27% at 4 days after CN injury (p=0.005). 2X SHH protein suppressed apoptosis 29% (p=0.003). Apoptosis increased 21% at 9 days after injury (p=0.014). Two SHH PA injections decreased apoptosis 22% at 9 days (p=0.021), while one SHH PA injection was indistinguishable from controls (p=0.830). SHH delivery to penis and CN decreased apoptosis 27% (p=0.0001).

Conclusions: Apoptosis suppression was similar in CN resection and crush models in response to SHH treatment. One SHH PA injection suppressed apoptosis until protein was depleted. Increasing the duration of SHH treatment, by a second SHH PA injection, suppressed apoptosis longer. Optimization of SHH PA delivery is essential for translation to prostatectomy patients to prevent ED.

Funding Source: NIH/NIDDK DK079184
Title: Sympathetic reinnervation of the urinary bladder using somatic donor nerves in a canine model of lower motoneuron lesioned bladder.

Authors: Mary F Barbe, Sandra Gomez-Amaya, Jennifer K Lewis, Stephen R Dachert, Matthew W Wood, Alan S Braverman, Michael R Ruggieri, Sr.

Affiliations: 1 Department of Anatomy and Cell Biology, Temple University School of Medicine, 3500 North Broad Street, Philadelphia, PA 19140; 2 Shriners Hospitals for Children of Philadelphia, 3551 North Broad Street, Philadelphia, PA 19140

Introduction/Objectives: Preganglionic sympathetic axons to pelvic viscera originate from lumbar preganglionic neurons and contribute to sympathetic chain ganglia and lumbar splanchnic nerves to the inferior mesenteric ganglia (many via the hypogastric nerve). Postganglionic sympathetic axons to the urinary bladder originate mostly from L7-S2 sympathetic chain ganglia and inferior mesenteric ganglion (1,2). We tested if after bladder decentralization and transfer of a lumbar originating nerve that postganglionic sympathetic axons will sprout from the lumbar nerves to reinnervate the urinary bladder.

Methods: A canine lower motoneuron lesioned bladder model was created by transecting sacral nerve roots that increased detrusor pressure using intraoperative electrical stimulation, and all roots caudal to S1. Female hounds underwent bladder decentralization and then femoral or genitofemoral nerve transfer (FNT, n=10, and GFNT, n=17, respectively) to the anterior vesicle branch of the pelvic nerve, or remained decentralized (n=3). Six sham/unoperated controls were included. Bladder emptying in animals without vesicostomies was accomplished by the Credé maneuver during the 8 month recovery period (242±6.2 days). Three weeks prior to euthanasia, a retrograde dye, Fluorogold, was injected into the bladder wall lateral to ureteral orifices. At euthanasia, T10 through coccygeal sympathetic ganglia and the inferior mesenteric ganglia were collected and examined for retrogradely labeled neuronal cell bodies. Multifactoral ANOVAs were performed (with the factors segment and group). The Bonferroni method of posthoc analysis for multiple comparisons with adjusted P values (adjusted down from p<0.05) was used to compare group differences.

Results: Increased numbers of fluorogold labeled cell bodies were observed in sympathetic ganglia of appropriate segments (mainly L4 and L5 in FNT animals; mainly L2 and L4 in GFNT animals), compared to sham/unoperated controls which showed fluorogold labeled cell bodies in S1-S3 sympathetic ganglia (p<0.01 each segment). Numbers of fluorogold labeled cells were also higher in sympathetic ganglia at vertebral levels L5 and L3 in FNT and GFNT animals, respectively, compared to decentralized controls (p<0.05 each). Low numbers of fluorogold labeled cells were visible in S1-S3 sympathetic ganglia of decentralized and FNT animals. The inferior mesenteric ganglia contained increased fluorogold labeled cells from the urinary bladder in both FNT and GFNT animals, compared to sham/unoperated controls (p<0.01 each).

Conclusions: Transfer of the femoral nerve to the anterior vesicle branch of the pelvic nerve lead to increased innervation of the bladder from lumbar postganglionic sympathetic ganglia, segments that did not contribute innervation in sham/unoperated controls. Functional consequences of this new innervation pattern should be explored in future studies.

Funding Source: NIH-NINDS NS070267
Title: Loss of nitric oxide-mediated inhibition of purine neurotransmitter release in the colon in the absence of interstitial cells of Cajal

Authors: Leonie Durnin¹, Andrea Lees¹, Sheerien Manzoor¹, Kent C. Sasse², Kenton M. Sanders¹ and Violeta N. Mutafova-Yambolieva¹*

Affiliations: ¹Department of Physiology and Cell Biology, University of Nevada School of Medicine; ²University of Nevada School of Medicine Minimally Invasive Solutions, Reno, NV, USA

Introduction/Objectives: Regulation of colonic motility depends upon the integrity of enteric inhibitory neurotransmission mediated by nitric oxide (NO), multiple purine neurotransmitters, and neuropeptides. Intramural interstitial cells of Cajal (ICC-IM) and platelet-derived growth factor receptor α-positive (PDGFRα+) cells are involved in generating responses to NO and purine neurotransmitters, respectively. Previous studies have reported reduced nitrergic neurotransmission and upregulation of purinergic signaling in gastrointestinal muscles from KitW/KitW-v (W/Wv) mice that display viable lesions in ICC-IM along the GI tract, including the colon. However, contributions of NO to these phenotypes have not been evaluated. The present study was undertaken to determine whether neural release of purines is altered in colons from W/Wv mice and to investigate the role of NO in these mechanisms.

Methods: We utilized small-chamber superfusion assays and high-performance liquid chromatography with fluorescence detection (HPLC-FLD) to measure the spontaneous and electrical field stimulation (EFS)-evoked release of nicotinamide adenine dinucleotide (NAD+)/ADP-ribose, uridine adenosine tetraphosphate (Up4A), adenosine 5′-triphosphate (ATP), and metabolites from the tunica muscularis of human and murine colons, and we tested drugs that modulate NO levels or blocked NO receptors.

Results: NO inhibited EFS-evoked release of NAD+/ADP-ribose, Up4A, and ATP in the colon via presynaptic neuromodulation. Colons from W/Wv, Nos1⁻/⁻, and Prkg1⁻/⁻ mice displayed augmented neural release of purines that was likely due to altered nitrergic neuromodulation. Colons from W/Wv mice demonstrated decreased nitrergic and increased purinergic relaxations in response to nerve stimulation. Extracellular purine metabolism was not altered in W/Wv colons. W/Wv mouse colons also demonstrated reduced Nos1 expression and reduced NO release, suggesting that NO available for prejuncƟonal inhibition of purine re-lease is likely reduced in these tissues.

Conclusions: NO mediates inhibition of purine release that may act to prevent “over-inhibition” of colonic muscles during increased motor neuron activation. Enhanced purinergic neurotransmission may compensate for the loss of nitrergic neurotransmission in muscles with partial loss of ICC. The interactions between nitrergic and purinergic neurotransmission in the colon provides novel insight into the role of neurotransmitters and effector cells in the neural regulation of gastrointestinal motility.

Funding Source: This study was supported by a grant from the National Institutes of Health, USA, DK 41315.
Title: Cholinergic excitatory motor responses in the colon are mediated through the Calcium-Activated Chloride Conductance Ano1

Authors: Sung Jin Hwang, Peter J. Blair, Jason Rock¹, David Pardo¹, Kenton M. Sanders & Sean M. Ward

Affiliations: Department of Physiology, University of Nevada, Reno School of Medicine, Reno Nevada, USA. ¹Department of Anatomy, University of California San Francisco, San Francisco, USA

Introduction: There is a growing body of evidence that gastrointestinal smooth muscle excitability is regulated by several different classes of interstitial cells (interstitial cells of Cajal (ICC) and PDGFRα(+) cells) that are electrically coupled to SMC. 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 gut and coordinate GI motility. Motor neurons innervate these three cell types in the SIP syncytium, and receptors, second messenger pathways, and ion channels in these cells mediate postjunctional responses. 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 the transient receptor protein channels Trpc4 and Trpc6. However, we have shown that cholinergic responses in the GI tract involve ICC and in their absence these motor responses are greatly diminished or absent.

Objectives: We sought to determine the conductance(s) responsible for cholinergic motor responses in the colon.

Methods: Cre-LoxP recombinase technology was utilized to determine the role of the calcium-activated chloride conductance, Ano1 in post-junctional motor responses in the mouse colon in a cell-specific manner (Kit⁺ ICC). c-KitCreERT2/+ (Kit-Cre) mice and Ano1f/f mice were crossed to generate c-KitCreERT2/+; Ano1f/f (Mutants) and c-KitCreERT2/+; Ano1f/+ (Controls) animals that were subsequently treated with Tamoxifen to induce Cre recombinase expression in ICC. Confocal microscopy was used to determine the cell type Cre expression was switched on. Intracellular microelectrode recordings were performed to determine changes in post-junctional neural responses to nerve stimulation in c-KitCreERT2/+ (Kit-Cre) mice and Ano1/+ mice to generate c-KitCreERT2/+; Ano1f/+ and c-KitCreERT2/+; Ano1f/+ animals mice treated with Tamoxifen using electrical field stimulation (EFS).

Results: Knock down of Ano1 in Kit⁺ ICC using the Cre/Lox P technology caused a marked reduction or loss of excitatory junction potentials (EJPs) in colonic muscles of c-KitCreERT2/+; Ano1f/+ in response to EFS compared to c-KitCreERT2/+; Ano1f/+ controls. These EJPs were atropine sensitive providing evidence that cholinergic muscarinic receptors were responsible for these excitatory responses.

Conclusions: These data provide evidence that Ano1 expressed in ICC and not smooth muscle cells is critically important for cholinergic excitatory post-junctional neural responses in colonic muscles.

Funding Sources: NIH PO1 41315 and RO1 DK57236.
PSA38

Title: Clarification of the innervation of genitourinary structures: a neuronal tracing study in female mongrel hound dogs

Authors: Mary F. Barbe, PhD\(^1\), Sandra M. Gomez-Amaya, DVM\(^1\), Neil S. Lamarre, PhD \(^2\), Danielle M. Salve-deo\(^1\), Michael Mazzei, MD\(^3\), Alan S. Braverman, Ph.D.\(^1\), Michael R. Ruggieri, Sr., PhD\(^1\)\(^4\)

Affiliations: \(^1\)Dept of Anatomy and Cell Biology, Temple University School of Medicine, 3500 North Broad Street, Philadelphia, PA 19140; \(^2\)Dept of Surgical Sciences, University of Wisconsin-Madison School of Veterinary Medicine, 2015 Linden Dr, Madison, WI 53706; \(^3\)Temple University Hospital, 3401 North Broad Street, Philadelphia; \(^4\)Shriners Hospitals for Children of Philadelphia, 3551 North Broad Street, Philadelphia.

Introduction/Objectives: Many studies focus on either afferent or efferent inputs (but not both), or on one structure of the genitourinary system. Only a few recent studies include information on inputs from sympathetic chain ganglia (SCG), and even fewer have examined the possibility of direct motor (autonomic or somatic) inputs from spinal cord ventral horns to genitourinary end organs. We sought to clarify origins of afferent and efferent information conveyed between the spinal cord, peripheral nervous system ganglia and genitourinary structures using retrograde and anterograde dye tracing methods.

Methods: Retrograde dyes were injected into the bladder wall, EUS and clitoris of 14 female mongrel dogs (Fluorogold, True blue, or Nuclear Yellow). Dorsal root ganglia (DRG), SCG, caudal mesenteric ganglion (CMG), pelvic plexus ganglia and spinal cord ventral horns were collected and examined for dye-labeled neuronal cell bodies. Detrusor muscle intramural ganglia were examined by injecting an anterograde dye (DiI) into the pelvic nerve’s anterior vesicle branch.

Results: Retrograde labeled cells were observed in several DRG, representative of afferent input from the bladder, EUS and clitoris (Table). Anterograde labeling revealed a number of intramural ganglia in the bladder wall after distal pelvic nerve labeling. Sympathetic efferents included: 1) labeled cells in the CMG primarily from the bladder, yet small numbers from the EUS and clitoris; 2) labeled cells in SCG primarily from the bladder (widespread) and more localized input from EUS and clitoris (Table); and 3) labeled cells in the intermediolateral cell column of thoracolumbar cord segments directly to the bladder and clitoris, a locale typically considered as sympathetic. Parasympathetic efferents included: 1) labeled neurons in pelvic plexus ganglia in bladder mesenteries; and 2) cells in lamina VII of sacral cord segments directly to the bladder and clitoris, a locale typically considered as sympathetic. Lastly, somatic (skeletal muscle) efferents to the EUS were evident as retrogradely labeled cells in sacral lamina IX cells.

<table>
<thead>
<tr>
<th>Bladder</th>
<th>EUS</th>
<th>Clitoris</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRG</td>
<td>L7-S2 (and low # in T1, L5, &amp; S3)</td>
<td>L7, S1 (and low # in L6)</td>
</tr>
<tr>
<td>SCG</td>
<td>L6-S2 (and low # in T10-L5, S3)</td>
<td>S1, S2 (and low # in L7,S3)</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>T10-L7 lamina VII (Sympathetic)</td>
<td>S1-S3 lamina IX (Somatic)</td>
</tr>
<tr>
<td></td>
<td>S1-S3 lamina VII (Parasympathetic)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Afferent and efferent inputs to genitourinary structures are complex, yet a clear knowledge is needed to understand dysfunction after spinal cord injury and mechanisms underlying chronic pain syndromes in this region.

Funding Source: NIH-NINDS NS070267
Title: The Use of Support Vector Machine in the Prediction of Stress Urinary Incontinence.

Authors: Brian M. Balog$^{1,2,3}$, and Haitao Zhao$^4$

Affiliations: Department of Biomedical Engineering, Cleveland Clinic, Cleveland, Ohio, USA$^1$, Advanced Platform Technology Rehabilitation R&D Center of Excellence, Louis Stokes Cleveland Veterans Medical Center, Cleveland, Ohio, USA$^2$, Department of Biology, University of Akron, Akron, Ohio, USA$^3$, Department of Computer Science, University of Akron, Akron, Ohio, USA$^4$

Introduction/Objectives: Stress Urinary Incontinence (SUI) is the involuntary leakage of urine due to an increase in abdominal pressure and it affects 30% of women over the age of 40. One of the primary risk factors is childbirth. The baby’s weight, head size and maternal age are just some of the variables clinicians can use to predict if women will develop stress urinary incontinence. Additionally, previous family history of SUI is another predictor for development suggesting a genetic role in development of SUI. A new method used to create predictive models is a support vector machine (SVM) use in the field of cancer biology. The purpose of the study was to determine if a SVM algorithm could construct a model that can improve the performance of predicting SUI compared with previous methods.

Methods: Data was obtained from the Pelvic Floor Disorder Network Childbirth and Pelvic symptoms Study (CAPS). Only information from the Urinary Incontinence and general data forms were used (e.g. maternal age, baby weight, head circumferences). We compared our models efficiency to a previously published model. Based on the data, we employ SVM algorithm to construct a model for predicting SUI. The basic idea of SVM is to find an optimal hyper-plane which can separate the data of one class from another class. In our study, we first divided the preprocessed data into two subsets, one is training data set, and the other one is testing data set. The testing data set was utilized to train an optimal model, that is, to find an optimal hyper-plane. The testing data set was employed to test the performance of the trained model. In order to obtain stable performance, we use 10 folds cross validation to train the model and to test its performance.

Results: An optimal hyper-plane was determined. The results indicate the accuracy of prediction is around 70 percent, which is a little better than that of previous methods at 69 percent.

Conclusions: The proposed method in this study can predict SUI. Further investigation is needed to determine if limitation of risk factors from the model can improve its performance.

Funding Source: There was no funding source for this project
Title: Spontaneous Ca\textsuperscript{2+} waves in mouse urethral smooth muscle visualized with a genetically encoded Ca\textsuperscript{2+} indicator in situ

Authors: Bernard T Drumm\textsuperscript{*1}, Sean M Ward\textsuperscript{1}, Kenton M Sanders\textsuperscript{1}

Affiliations: \textsuperscript{1}Department of Physiology & Cell Biology, University of Nevada School of Medicine, Reno NV, USA

Introduction/Objectives: The urethra generates myogenic tone during bladder filling to maintain continence. There are few visual studies of urethra motor activity and the specific Ca\textsuperscript{2+} signalling of urethral smooth muscle is relatively unknown. In the present study, we sought to characterize the pattern of smooth muscle cell activation in urethra utilizing Ca\textsuperscript{2+} imaging with a genetically encoded Ca\textsuperscript{2+} reporter, GCaMP3.

Methods: Ca\textsuperscript{2+} signals in mouse urethra were imaged using GCaMP3, activated by a smooth muscle heavy chain promoter. The urethra was excised and urothelium layer removed by sharp dissection. Ca\textsuperscript{2+} signals were recorded in situ using an upright fluorescent microscope. Enzymatically dispersed mouse urethral smooth muscle cells with an eGFP tag were purified for qPCR analysis using fluorescence-activated cell sorting.

Results: Urethral smooth muscle cells fired spontaneous intracellular Ca\textsuperscript{2+} waves. Ca\textsuperscript{2+} waves did not propagate from cell to cell and activity in individual cells was not correlated with adjacent cells. Ca\textsuperscript{2+} waves were significantly increased in frequency, amplitude, duration and spatial spread by the a1a agonist phenylephrine and abolished by the guanylate cyclase donor NONATE. Ca\textsuperscript{2+} waves originated from both Ca\textsuperscript{2+} influx and Ca\textsuperscript{2+} release from intracellular stores as removal of extracellular Ca\textsuperscript{2+} and blocking the sarcoplasmic Ca\textsuperscript{2+} - ATPase abolished Ca\textsuperscript{2+} waves. Ca\textsuperscript{2+} waves were insensitive to the L-type Ca\textsuperscript{2+} channel blocker nifedipine but were inhibited by blockers of T-type Ca\textsuperscript{2+} channels (NNC 55-0396 and TTA-A2) as well as blockers of ryanodine and IP\textsubscript{3} receptors (tetracaine and 2-APB). qPCR revealed that urethral smooth muscle cells expressed the ER Ca\textsuperscript{2+} release channels IP\textsubscript{3}R1,2 and RyR1-3 as well as the voltage dependent Ca\textsuperscript{2+} influx channels Cacna1s, Cacna1c, Cacna1d, Cacna1f and Cacna1h.

Conclusions: Urethral smooth muscle cells fire spontaneous Ca\textsuperscript{2+} waves which are modulated by both adrenergic and nitrergic inputs. These Ca\textsuperscript{2+} waves rely on Ca\textsuperscript{2+} influx from a non-L type Ca\textsuperscript{2+} influx mechanism and release of Ca\textsuperscript{2+} from intracellular stores for their generation.

Funding Sources: R01 DK-091336, P01 DK41315
Localization of Neuromuscular Nicotinic Receptors in the Functionally Reinnervated Canine Bladder after Prolonged Decentralization

**Title:** Localization of Neuromuscular Nicotinic Receptors in the Functionally Reinnervated Canine Bladder after Prolonged Decentralization

**Authors:** Nagat Frara¹, Alan S. Braverman¹, Danielle M. Salvadeo¹, Ekta Tiwari¹, Mary F. Barbe¹, Michael R. Ruggieri, Sr.¹,²

**Affiliation:** ¹Department of Anatomy and Cell Biology, Lewis Katz School of Medicine, Temple University, Philadelphia, PA, USA; ²Shiners Hospitals for Children of Philadelphia, Philadelphia, PA, USA

**Introduction/Objectives:** We previously found that intravenous succinylcholine, a depolarizing neuromuscular nicotinic receptors blocker, prevents bladder contractions induced by new neuronal pathways established by nerve transfer in decentralized dogs. We studied the detrusor pressure response in vivo and contractile response of bladder smooth muscle strips in vitro from sham, decentralized and reinnervated animals to localize the neuromuscular nicotinic receptors involved.

**Methods:** Three groups of female mongrel hound dogs were used: sham (N=4), 12-month decentralized (N=3) and 6-month reinnervated (N=3). Decentralization was created by bilateral transection of all spinal roots caudal to L7, including the dorsal roots of L7 and the hypogastric nerves. Reinnervation was created by bilateral transfer of the obturator nerve to anterior vesical branches of the pelvic nerve. Two-way ANOVAs and Sidak post-hoc tests were used to determine group differences.

**Results:** In reinnervated dogs, blockade of neuromuscular nicotinic receptor with intravenous injections of the competitive antagonist atracurium besylate significantly reduced the increase in detrusor pressure induced by electrical stimulation of the transferred obturator nerve (L1 or L2). Atracurium did not block the increase in pressure induced by stimulation of sacral nerve roots in sham-operated controls. In vitro, neither the competitive neuromuscular nicotinic receptor antagonist d-tubocurarine nor the ganglionic antagonist hexamethonium inhibited electric field stimulation (EFS)-induced contractions of reinnervated or sham-operated control bladder strips. No contractile response was elicited in the presence of 1 μM tetrodotoxin (TTX) across groups. Similarly, EFS-evoked contractions were strongly reduced by 10 μM alpha, beta-methylene ATP (α,β-mATP) and 1 μM atropine in all groups relative to the vehicle (water).

**Conclusions:** In vivo blockade of nerve-evoked bladder pressure by atracurium in the reinnervated, but not sham operated controls, suggests that neuromuscular nicotinic receptors become involved in bladder contractions induced by the new neuronal pathway. Because d-tubocurarine did not block in-vitro contractions induced by EFS in the reinnervated bladders, the neuromuscular nicotinic receptors involved in the new neuronal pathway must not be located in the bladder muscle or intramural ganglia and therefore, are likely in preganglionic neurons. TTX blockade validates that EFS-induced contractions at all frequencies were nerve-evoked. Both muscarinic and purinergic components contributed similarly to neurotransmission based on response to blockade of nerve evoked muscle strip contractions with a combination of atropine and α,β-mATP.

**Funding Source:** NIH-NINDS NS070267
Title: Cyclophosphamide-induced Overactive Bladder via Downregulation of Relaxation Factors in Detrusor PDGFRα⁺ Cells

Authors: Haeyeong Lee PhD, Byoung H. Koh BS, Robert D. Corrigan BS, Lauren E. Peri BS, Kenton M. Sanders PhD, Toby C. Chai MD and Sang Don Koh MD PhD.

Affiliation: ¹Department of Physiology & Cell Biology, University of Nevada Reno, School of Medicine, Reno, NV, USA; ²Department of Urology, School of Medicine, Yale University, New Haven, CT, USA.

Introduction/Objectives: Morphology and functional role of PDGFRα⁺ cells have been recently characterized in the detrusor muscle layer. Detrusor relaxation is caused by activation of small conductance Ca²⁺ activated−K⁺ (SK) channels and purinergic inhibitory responses in detrusor PDGFRα⁺ cells. Loss of PDGFRα⁺ cells or alteration of P2Y receptors and SK channels will affect detrusor excitability. Cyclophosphamide (CYP)−treated animals exhibited overactive bladder (OAB). We hypothesized that the downregulation of P2Y receptors and/or SK channels in PDGFRα⁺ cells will display the phenotype of CYP−induced OAB.

Methods: CYP was injected intraperitoneally in PDGFRα⁺/eGFP and SMC/eGFP mice. We harvested the detrusor muscle without urothelium and disperse the cells for the fluorescence activated cell sorting (FACS). Sorted PDGFRα⁺ cells and smooth muscle cells (SMCs) were used for molecular study to compare the changes in transcripts between CYP−injected and control group. Transcripts were examined included; Pdgfα, P2ry1, P2ry2, P2ry4, Kcnn1, Kcnn2, Kcnn3 and inflammation marker (Il−6). Immunohistochemistry, mechanical contractility and ex vivo cystometry were also performed.

Results: Quantitative analysis of PCR revealed that CYP−injected detrusor muscle increased transcriptional expression of Il−6, but decreased the expression of Pdgfα. Transcriptional changes in CYP−injected sorted PDGFRα⁺ cells from PDGFRα⁺/eGFP mice showed Pdgfα, Kcnn3 (SK3), P2ry1, P2ry2 and P2ry4 genes were decreased compared with saline−injected control. Sorted SMCs from SMC/eGFP mice did not show significant expression of those genes and no detectable changes. Immunohistochemistry showed SK3 in PDGFRα immunoreactivity was downregulated in CYP−injected detrusor muscle. Apamin (a SK blocker) sensitivity on spontaneous contractile activity was decreased in CYP−injected mice compared to saline−injected mice. In ex vivo cystometry, increased spontaneous non−voiding contractions and less apamin sensitivity were observed in CYP−injected mice.

Conclusions: These findings are the first report to investigate the role of PDGFRα⁺ cells in relation to OAB mechanisms. In conclusion, we found that CYP−induced OAB is resulted from down regulation of PDGFRα, P2Y receptors and SK channels in CYP−injected bladder. These results provide novel mechanisms of functional role of PDGFRα⁺ cells on OAB.

Funding Sources: Supported by NIDDK, RO1 DK098388 and Urology Care Foundation Research Scholar Award (Interstitial Cystitis Association)
Title: Comparison of Bladder Volumes between 2D and 3D Ultrasound Calculations and Urodynamic Measurements in Women with Overactive Bladder (OAB)

Authors: Rachel J Bernardo¹, Anna S Nagle PhD*², Adam P Klausner MD³, & John E Speich PhD²

Affiliations: ¹Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA. ²Department of Mechanical & Nuclear Engineering, Virginia Commonwealth University, Richmond, VA. ³Department of Surgery/Division of Urology, Virginia Commonwealth University School of Medicine, Richmond, VA.

Introduction/Objectives: Various methods are currently available to non-invasively quantify bladder volume. The goal of this project was to determine the most accurate method of quantifying bladder volume using 2D and 3D ultrasound techniques during UD.

Methods: Nine female participants with OAB underwent an extended urodynamics procedure (Laborie Aquarius XT) while ultrasound images of the bladder were obtained using a 3D 6MHz transabdominal probe (GE Voluson E8). The bladder was filled with saline at a rate of 10% bladder capacity (based on an initial clinical fill) per minute while ultrasound images were captured once per minute. Bladder volume was estimated from 2D cross-sectional images in the sagittal and transverse planes assuming an ellipsoid geometry (Eqn 1, \( V_{\text{spheroid}} \)), assuming a shape in between an ellipsoid and a cube (Eqn 2, \( V_{\text{Bih}} \) by Bih et. al. 1998), and from the 3D ultrasound data obtained by tracing the bladder outline in six planes with GE’s 4D View software (\( V_{3D} \)).

\[
V_{\text{spheroid}} = \frac{\pi}{6}(W \times H \times D) \quad \text{Eqn. 1}
\]

\[
V_{\text{Bih}} = 0.72 \times W \times H \times D = 1.375 \times V_{\text{spheroid}} \quad \text{Eqn. 2}
\]

In Equations 1 and 2, \( W \) is the width (horizontal diameter) and \( H \) is the height (vertical diameter) in the sagittal direction and \( D \) is the depth in the transverse direction (horizontal diameter).

Results: \( V_{\text{spheroid}} \) was significantly lower than infused volume (\( V_{H2O} \)) when compared by a paired t-test. \( V_{\text{Bih}} \) and \( V_{3D} \) tended to be slightly larger than \( V_{H2O} \), not significantly (Fig. 1).

Conclusions: The bladder shape cannot be assumed to be an ellipsoid in patients with OAB. Tracing the perimeter in several 3D imaging planes better accounts for the non-uniform geometry, providing a more accurate volume measurement. Volumes estimated by \( V_{\text{Bih}} \) or by tracing the bladder in 3D were not significantly different from \( V_{H2O} \), demonstrating that these are the most accurate methods of non-invasive assessment of bladder volume.

Funding Sources: Support provided by NIH R01DK101719, VCU Presidential Research Quest Fund, and VCU Dean’s Undergraduate Research Initiative.

Figure 1. Comparison of volume methods at 100% bladder capacity. All image based methods were compared to infused volume (\( V_{H2O} \)) by paired t-test. The volumes generated by the 2D spheroid method were significantly less than the infused volume (denoted by a star).
Title: Pharmacological Activation of Individual KCNQ Channel Subtypes in Detrusor Smooth Muscle Represents a Promising Novel Approach for Overactive Bladder Treatment

Authors: Aaron Provence, Damiano Angoli, and Georgi V. Petkov

Affiliations: Department of Drug Discovery and Biomedical Sciences, University of South Carolina, Columbia, SC-29208, USA

Introduction/Objective: Our recent studies have demonstrated voltage-gated KCNQ channels (KCNQ1-KCNQ5) as key regulators of detrusor smooth muscle (DSM) function. Despite emerging developments, the physiological role of individual KCNQ channel subtypes remains less clear. Here, we utilized the novel compound ML-213, a potent activator of KCNQ2, KCNQ4, and KCNQ5 channels, to elucidate their physiological roles in guinea pig DSM function.

Methods: Using isometric DSM tension recordings, Ca^{2+} imaging, and amphotericin-B perforated patch-clamp electrophysiology, we elucidated the role of ML-213-sensitive KCNQ channels in regulating DSM excitability and contractility.

Results: ML-213 concentration-dependently (100 nM-30 µM) inhibited spontaneous phasic, pharmacologically-induced, and nerve-evoked contractions in DSM isolated strips. ML-213 (10 µM) decreased the global intracellular Ca^{2+} concentrations in DSM isolated strips, effects blocked by the L-type voltage-gated Ca^{2+} (Ca_v) channel inhibitor nifedipine (1 µM) and the KCNQ1-KCNQ5 channel inhibitor XE991 (10 µM). These data suggest that ML-213 decreases the global intracellular Ca^{2+} concentration by inhibiting L-type Ca_v channels through an indirect mechanism downstream from KCNQ channel activation. In addition, ML-213 hyperpolarized the cell membrane potential and inhibited spontaneous action potentials in DSM cells, effects reversible by washout. We next aimed to examine the effects of ML-213 on whole cell KCNQ currents. To isolate KCNQ currents, the bath solution contained the large conductance voltage- and Ca^{2+}-activated K^+ channel inhibitor paxilline (1 µM) and gadolinium chloride (GdCl_3, 50 µM), which blocks L-type Ca_v channels and non-selective cation channels. Under these experimental conditions, ML-213 (10 µM) enhanced whole cell KCNQ currents. These findings suggest that the modulation of K^+ transport through ML-213-sensitive KCNQ channels underlies ML-213-induced cell membrane hyperpolarization to decrease the global intracellular Ca^{2+} concentration and DSM contractility.

Conclusions: These data using the novel compound ML-213, suggest that KCNQ2, KCNQ4-, and KCNQ5-containing channels are essential regulators of the excitability, intracellular Ca^{2+} concentration, and contractility of DSM by virtue of their control of the membrane potential. Moreover, these new findings provide a foundational basis for future investigations on KCNQ channel functional roles in human DSM excitability and contractility to confirm their potential as novel therapeutic targets for overactive bladder.

Source of Funding: Supported by NIH grant R01-DK106964 to Georgi V. Petkov and F31-DK104528 to Aaron Provence.
Title: Decentralization reduces nicotinic receptor-mediated canine bladder contractions in vitro

Authors: Danielle M. Salvadeo¹, Nagat Frara¹, Alan S. Braverman¹, Mary F. Barbe¹, Michael R. Ruggieri, Sr. ¹²³

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

Introduction/Objectives: Bladder function depends upon several complex signaling pathways that induce either contraction or relaxation. We performed nerve re-routing surgery on bladder of decentralized dogs (reinnervated) as a model with the goal of restoring bladder function in spinal cord injured patients. The neuromuscular nicotinic receptor blocker, succinylcholine blocks spinal root-stimulated bladder contraction in vivo in reinnervated dogs, but not in sham-operated dogs. Our lab explored the function and location of nicotinic receptors involved in bladder contraction, with emphasis on their possible role in the release of other neurotransmitters such as acetylcholine or ATP in sham-operated, 12 month-decentralized and immediate-reinnervated bladders.

Methods: Smooth muscle strips (mucosa denuded) were isolated from the region just rostral to the trigone and suspended in muscle baths. Strips were ranked based on their contractile response to a 3-min exposure to 120 mM KCl and sorted so that the average response in each group was equal. Strips were incubated with either 1 µM atropine (ATR), 1 µM tetrodotoxin (TTX), 10 µM alpha, beta-methylene ATP (α,β-ATP) or vehicle (water) and then induced to contract with the nicotinic receptor agonists 1,1 dimethyl-4-phenyl-piperazinium iodide (DMPP, 100 µM), TC2559 (100 µM) or epibatidine (10 µM) or 1 mM nicotine itself.

Results: The DMPP-induced contraction was not different between sham, reinnervated or decentralized bladders (11%, 3.3%, and 3.6% of KCl contraction respectively). While the epibatidine-induced contraction in shams was not different relative to that in the reinnervated (41% vs. 27% KCl respectively), it was significantly greater than that in decentralized bladders (13% of KCl). TC2559 did not induce bladder contractions. Nicotine-induced contractions in sham-operated controls were 16% of KCl. ATR completely blocked nicotine-induced contraction while α,β-ATP had no statistically significant effect in shams. TTX had no significant inhibitory effect on DMPP, epibatidine or nicotine-induced contraction in any group.

Conclusions: Nicotinic receptors mediate contraction in sham, reinnervated and decentralized bladders. This nicotinic receptor-mediated contraction is decreased after decentralization. TTX does not block nicotinic receptor-mediated contractions, indicating that action potentials are not required to induce contraction. In sham-operated dog bladders, the nicotine-induced contraction is blocked by ATR, suggesting that these nicotinic receptors are located on cholinergic nerve terminals and induce the release of acetylcholine, which activates muscarinic receptors on the smooth muscle.

Funding Source: NIH-NINDS NS070267
Title: In-vivo hypogastric nerve electrical stimulation contracts the canine detrusor.

Authors: Ekta Tiwari¹, Danielle M. Salvadeo², Luke V. Musser², Matthew W. Wood², Alan S. Braverman², Mary F. Barbe², Michael R. Ruggieri, Sr.,¹,²,³

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

Introduction/Objectives: Our goal was to determine whether electrical stimulation of the hypogastric nerve in dogs with intact bladder innervation results in contraction of the detrusor muscle of the bladder. The effects of the anesthetics isoflurane versus propofol on detrusor pressure with hypogastric nerve stimulation were compared.

Methods: A total of 14 female mixed-breed hounds with intact bladder innervation, body mass of 20-25 Kg and 6-8 months of age were used. Maximum changes in detrusor pressures were determined following electrical stimulation (3-10mA, 20Hz) of the hypogastric nerves under isoflurane inhalation anesthesia at 1-3% mean alveolar concentration in oxygen (n=14). Five of these same dogs were then transitioned to propofol anesthesia administered intravenously by continuous-rate infusion (0.3-0.5 mg/kg/min). Detrusor pressures were recorded at a sampling rate of 4/s using PowerLab software (AD Instruments) and displayed using LabChart. Following electrical stimulation, hypogastric nerves were harvested from five dogs undergoing decentralization for other experiments. These nerves were cryosectioned and examined for expression of the adrenergic marker enzyme tyrosine hydroxylase (present in sympathetic peripheral nerves) using immunohistochemical methods.

Results: Electrical stimulation of the hypogastric nerve caused an increase in detrusor pressure (2 to 9 cm H₂O) under isoflurane anesthesia in 11 of 14 dogs. Two of the 5 dogs tested under propofol anesthesia showed an increase in detrusor pressure after switching from isoflurane to propofol, another showed no change, and the remaining two showed an absence of bladder contractions in response to hypogastric nerve stimulation under either anesthetic. Each collected hypogastric nerve showed positive tyrosine hydroxylase immunostaining, confirming that the nerves contain sympathetic fibers.

Conclusions: These results indicate that the neuronally intact canine detrusor muscle contracts during hypogastric nerve stimulation under both isoflurane and propofol anesthesia. These results confirm findings by de Groat and colleagues in cats (1,2) and by Elmer in rats showing that electrical stimulation of hypogastric nerves in animals with intact bladder innervation elicits low-amplitude bladder contractions.

Funding Source: NIH-NINDS NS070267

Literature cited:

Title: Monitoring nerve activity during bladder filling in a rat model.

Authors: Ekta Tiwari¹, Michel A. Lemay², Alan S. Braverman³, Iyad Obeid¹, Mary F. Barbe³, Michael R. Ruggieri, Sr.¹,³,⁴

Affiliations: ¹Department of Electrical and Computer Engineering, ²Department of Bioengineering, College of Engineering, ³Department of Anatomy and Cell Biology, School of Medicine, Temple University, and ⁴Shriners Hospitals for Children, Philadelphia, PA, USA.

Introduction/Objectives: Surgical rerouting of neuronal pathways may allow functional reinnervation of the bladder. We aim to develop techniques to monitor afferent (sensory) nerve activity during bladder filling in normal intact bladders for eventual application to monitoring sensory reinnervation of the bladder following nerve transfer.

Methods: Electroneurogram recordings were performed in anesthetized rats under isoflurane (1-2% induction dose, inhalation) of: 1) sciatic nerves during stimulation of the hindpaw with Semmes-Weinstein monofilaments of varying forces (10g-300g), and 2) bladder nerves during bladder filling with infusion rate of 0.5ml/min, in acute experiments. Bipolar cuff electrodes were wrapped around the sciatic nerve (n=7) and ureter (and associated bladder nerves) proximal to the bladder wall (n=7), to record sciatic and bladder nerve discharges, respectively. The sciatic nerve was transected between the spinal cord and the electrode, to eliminate efferent nerve signals and record afferent fibers discharge only with hindpaw stimulation. Whereas, recordings were made of the discharges of both afferent and efferent fibers from bladder nerves during bladder filling. All recordings were performed using a low noise amplifier (SR560, filtered 300Hz-10kHz, gain x10k), sampled at 20kHz using PowerLab software (AD Instruments) and displayed using LabChart software. Bladder pressure was also recorded during filling.

Results: Sciatic nerve recordings consistently showed increased afferent fibers discharge with increased size of monofilament used to stimulate the hindpaw, with the highest discharge observed with the 300g monofilament and lowest with 10g. In contrast, recording from bladder nerves showed that combined afferent and efferent discharges increased substantially in response to bladder filling in 2 of 7 rats, and increased moderately in 2 other rats. However, there was no response in the remaining rats, perhaps due to nerve damage during cuff placement.

Conclusions: We found an increase in afferent discharges during paw stimulation with increased monofilaments sizes. Also, recordings from the cuff around the ureter-bladder nerve complex revealed that the afferent and efferent discharges coincided with an increase in bladder pressure during bladder filling. We could not isolate single fibers (units) from these whole nerve recordings, thus we cannot report on individual nerve fiber activity. However, based on these results, we conclude that the present technique used to record nerve activity in the rat model may be suitable to record nerve activity during bladder filling in canines with surgically rerouted neural pathways.

Funding Source: NIH 1R01NS070267
Title: The Effects of Myrbetriq on Detrusor Overactivity associated with Suprasacral Spinal Cord Injury (SCI) in Rats

Authors: Jillene M. Brooks¹, Danielle J. Degoski¹, Matthew O. Fraser²

Affiliations: ¹Institute for Medical Research, Durham, NC; ²Duke University and Durham Veterans Affairs Medical Centers, Durham, NC

Introduction/Objectives: β3-adrenoceptor agonists (BARA) represent a novel mechanism of action for direct relaxation of urinary bladder smooth muscle. Myrbetriq (MYR) is an FDA approved BARA developed by Astellas Pharma, and has proven to be very useful for treating overactive bladder. Preliminary data from this laboratory demonstrated a remarkable effect of a rat-specific BARA, CL-316,243 (CL), on the hallmark attributes of neurogenic bladder subsequent to SCI. This included an increase in true bladder capacity (TBC), a decrease in the number and amplitude of non-voiding contractions (NVC) and an increased filling compliance (C). The current report reflects a formalized preclinical study of both the rat specific BARA, CL, and MYR, in order to provide preclinical support for utilization of MYR in treating SCI patients with neurogenic detrusor overactivity.

Methods: Female rats (4 weeks post-SCI at T9-10, n=43, 10-11/group) were anesthetized with isoflurane and fitted with femoral vein, ureteral diversion and transvesical catheters. The animals were mounted in Ballman restraint cages to which they had been previously acclimated. Conscious cystometry was performed before and after 3 repeated vehicles (Veh 1-3) and 3 escalating 1/2 log doses of either CL, MYR, or their paired repeated vehicle controls (Veh 4-6) at 30 minute intervals. See Figure for testing scheme protocol. Data were analyzed using ANOVA with repeated measures. P<0.05 was considered significant.

Results: Both CL and MYR significantly increased TBC and NVC count, MYR decreased maximal NVC amplitude, and CL increased C, relative to their respective repeated vehicle controls.

Conclusions: The results of these studies support the use BARA for the treatment of NDO secondary to SCI, as at least one of the drugs tested was able to distinguish themselves from their repeated vehicle control groups as having a positive effect for each of the four measures.

Funding Source: This work was supported by an IIR grant from Astellas.