Friday 27th March, 2015

Welcome reception (hors d’oeuvres, drinks; 6:00-7:00): Foyer SAHMRI

**Plenary Session** (7:00-8:00pm): SAHMRI auditorium

**Chair:** Christine Feinle-Bisset

7:00-7:10pm  
Steve Wesselingh  
Welcome address

7:10-8:00pm  
Professor David Grundy  
The University of Sheffield  
*What has sensory biology told us about gut disease?*

Saturday 28th March, 2015

**Scientific session 1** (9:00-10:30am): Gastrointestinal motility and transit

**Chair:** John Furness

9:00-9:30am  
Rebecca Burgell  
Alfred Hospital and Monash University  
*Anorectal sensory and motor dysfunction*

9:30-10:00am  
Marcello Costa  
Flinders University  
*Recent progress in large bowel motility*

10:00-10:15am  
Peter Wu  
St George Hospital, University of NSW  
*Endolumenal functional lumen imaging probe (EndoFLIP) provides a valid and sensitive direct measurement of the upper oesophageal sphincter compliance in patients with radiotherapy-related pharyngeal dysphagia*

10:15-10:30am  
John Arkwright  
Flinders University  
*Quantifying phase II patterns of the migrating motor complex in the human small bowel using high resolution fibre optic manometry*

Morning tea, SAHMRI foyer: 10:30-11:00am
Scientific session 2 (11:00am-12:30pm): Young investigator session

Chair: Christine Feinle-Bisset

11:00-11:18am
Stephen Kentish
University of Adelaide and South Australia Health and Medical Research Institute
Obesity disrupts 17β-estradiol signalling in gastric vagal afferents

11:18-11:36am
Hui Li
University of Adelaide and South Australia Health and Medical Research Institute
Modulatory effect of apelin on gastric vagal afferents mechanosensitivity in different feeding status

11:36-11:54am
Kristen Farrell
University of Newcastle and Hunter Medical Research Institute
In vivo characterisation of spinal cord neurons with visceral inputs

11:54-12:12pm
Teng Zhang
St George Hospital and University of New South Wales
Biomechanics of deglutitive dysfunction following total laryngectomy

12:12-12:30pm
Luke Grundy
The University of Adelaide and South Australia Health and Medical Research Institute
Ciguatoxin potently activates colonic nociceptive pathways with greatest efficacy in a model of chronic visceral hypersensitivity

Lunch and poster session, SAHMRI foyer: 12:30-2:00pm

Scientific session 3 (2:00-3:30pm): Pharyng-o-oesophageal physiology and pathophysiology

Chair: Ian Cook

2:00-2:40pm
Jenny Myers
University of Adelaide and Royal Adelaide Hospital
Charles Cock
Repatriation General Hospital and Flinders University
Demonstration: Pharyng-o-oesophageal physiology in vivo

2:40-3:00pm
Michal Szczesniak
University of New South Wales and St George Hospital
Video-HRiM diagnosis of pharyngeal dysfunction

3:00-3:30pm
Richard Holloway
Royal Adelaide Hospital
The Chicago Classification of oesophageal motility version 3.0
Afternoon tea, SAHMRI foyer: 3:30-4:00pm

**Scientific session 4** (4:00-5:30pm): Sensory innervation of the gastrointestinal tract

**Chair:** Simon Brookes

4:00-4:30pm  
**David Grundy**  
University of Sheffield  
*Extrinsic sensory neurons, the interface between the gut and the brain*

4:30-5:00pm  
**Joel Bornstein**  
University of Melbourne  
*Intrinsic sensory neurons, not just primary afferents*

5:00-5:15pm  
**Amanda Page**  
University of Adelaide, South Australia Health and Medical Research Institute and Royal Adelaide Hospital  
*High fat diet-induced obesity disrupts circadian variations in gastric vagal afferent satiety signals in mice*

5:15-5:30pm  
**Stuart Brierley**  
University of Adelaide and South Australia Health and Medical Research Institute  
*A visceral representation of itch: Identification 'itch-specific' pruritogenic mechanisms within colonic sensory pathways*

Annual General Meeting: 5:30-6:30pm

Banquet, Adelaide Convention Centre 7:00-11:00pm

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**Sunday 29th March, 2015**

**Scientific session 5** (9:00am-10:30am): Inflammation and inflammatory bowel disorders

**Chair:** Richard Holloway

9:00-9:30am  
**Sam Costello**  
The Queen Elizabeth Hospital and Royal Adelaide Hospital  
*Faecal microbiota transplant in ulcerative colitis*
9:30-10:00am  Patrick Hughes  
University of Adelaide  
Inflammation and sensory function

10:00-10:15am  Kulmira Nurgali  
Victoria University  
Mesenchymal stem cell-based treatments for enteric neuropathy associated with colitis

10:15-10:30am  Jesse Di Cello  
Monash Institute of Pharmaceutical Sciences  
Expression and distribution of the delta opioid receptor in acute colitis

Morning tea, SAHMRI foyer: 10:30-11:00am

Debate (11:00-12:30am): Pharmacotherapy/diet/psychotherapy for the treatment of IBS

Chair: Gerald Holtmann

11:00 – 11:20am  Nick Talley  
University of Newcastle  
Irritable bowel syndrome (IBS): Pharmacotherapy versus Diet versus Psychological Treatments

11:20-11:40am  Peter Gibson  
Alfred Hospital and Monash University  
Diet

11:40-12:00pm  Jim Kantidakis  
The Gut Centre  
The role of psychological therapies in functional GI disorders

12:00-12:30pm  Open discussion

Meeting concludes: 12:30pm
Session: Clinical/Human Studies

Poster #1 Lauren D. Dughetti, Julie Jordan-Ely, Kyla Dobson, Lefteris Stathopoulos, Marcelo Leal, Tony Catto-Smith, John M. Hutson, Bridget R. Southwell

Combining existing laxatives to disimpact the really hard cases: Producing effective colonic motility in children with chronic constipation and palpable faecaloma

Poster #2 Julie Jordan-Ely, John M. Hutson, Bridget R. Southwell

Disimpaction of children with constipation in a suburban clinic using polyethylene glycol and sodium picosulphate

Poster #3 Bridget R. Southwell, Andre Tan, John M. Hutson

Development of rhythm.IC: A medical device to increase bowel motility and treat constipation

Poster #4 Bridget R. Southwell, Julie Jordan-Ely, John M. Hutson, Geoff Hebbard

Transcutaneous electrical stimulation across the abdomen improves symptoms in adults with gastroparesis: A pilot study

Poster #5 Andrea M. Harrington, Joel Castro, Richard L. Young, Caroline Kurtz, Ada Silos-Santiago, Nam Nguyen, Jane M. Andrews, Stuart M. Brierley

Distinct alterations in the guanylate cyclase-C/cGMP pathway are evident across different subtypes of irritable bowel syndrome patients

Poster #6 Ryash Vather, Greg O’Grady, Leo K. Cheng, Lukasz Wiklendt, David Rowbotham, Ian P. Bissett, Phil G. Dinning

Preserved colonic meal response and functional evidence for anastomotic nerve regeneration in patients with normal bowel function following anterior resection

Poster #7 Sophie Wessel, Iian J.N. Koppen, Marc Benninga, Lukasz Wiklendt, Phil G. Dinning

Utilising high-resolution colonic manometry to quantify dysmotility in children with slow transit constipation

Poster #8 Charles Cock, Stamatiki Kritas, Laura Besanko, Carly Burgstad, Alison Thompson, Richard Heddle, Robert J. Fraser, Taher Omari

Integrated relaxation pressure (IRP4) increases during viscous swallowing in aged subjects

Poster #9 Charles Cock, Stamatiki Kritas, Carly Burgstad, Alison Thompson Laura Besanko, Richard Heddle, Robert J. Fraser Taher Omari

Upper esophageal sphincter (UES) maximum admittance identifies dysphagia due to neuromuscular and structural pathology

Poster #10 Rochelle Botten, Addolorata DiMatteo, Kanan Sharma, Phil G. Dinning, Braden Higgs, Robert Fraser, Eric Yeoh

Endorectal balloon (ERB) during image guided radiation therapy (igrt) for prostate carcinoma (CaP) reduces radiation proctitis at 2 years
Session: **ENS, cells, molecules**

Poster #11  Rachel McQuade, Vanesa Stojanovska, Simona Carbone, Ahmed Rahman, Kulmira Nurgali

*Enteric neuropathy induced by anti-cancer chemotherapeutic drug Oxaliplatin*

Poster #12  Lih En Tiah, Nigel W Bunnett, Holly R. Yeatman, Daniel P. Poole, Meritxell Canals, Michelle L. Halls

*The impact of internalisation on compartmentalised signalling of the delta-opioid receptor*

Poster #13  Jerry Zhou, Melinda Lam, Aniko Huizer-Pajkos, Sarah Hilmer, Vincent Ho

*Effects of polypharmacy treatment on interstitial cell of Cajal and neuronal networks in mice gastrointestinal tract*

Poster #14  Dongcheng Zhang, Benjamin N. Rollo, Lincon A. Stamp, Trevelyan R. Menheniott, Lefteris Stathopoulos, Mark Denham, Mirella Dottori, Sebastian K. King, John M. Hutson, and Donald F. Newgreen

*Enteric neural cells from Hirschsprung disease patients form ganglia autologously in aneuronal colon muscle tissue*

Poster #15  Laura Edgington-Mitchell, Nigel W. Bunnett, & Matthew Bogyo

*Characterizing the role of the cysteine protease legumain during caerulein-induced pancreatitis using activity-based probes*

Poster #16  Marcello Costa, Pam Simpson, Simona Carbone, Lukasz Wiklendt, Phil G. Dinning, Nick J. Spencer, and Simon J.H. Brookes

*Lidocaine-insensitive enteric inhibitory motor neurons are involved with migrating motor complexes in the guinea-pig colon*

Poster #17  Nick J. Spencer, Lee Travis, Timothy J Hibberd, Marcello Costa, Simon J.H. Brookes, Phil G. Dinning, Lukasz Wiklendt

*High resolution neuronal imaging reveals a novel oscillatory firing mechanism in the enteric nervous system that underlies migrating complex generation*

Poster #18  Gayathri K. Balasuriya, Elisa L. Hill-Yardin, Joel C. Bornstein

*Sexual dimorphic effects of cholera toxin in colonic motility are mediated via estrogens and serotonin in female C57BL/6 mice*

Session: **Food intake, appetite, metabolism**

Poster #19  Jessica Biesiekierski, Jan Tack

*Assessment of gastric accommodation by intragastric pressure measurement during different nutrient drink infusions*

Poster #20  Nada Cvijanovic, Nicole J. Isaacs, Christopher K. Rayner, Christine Feinle-Bisset, Richard L. Young, Tanya J. Little

*Relationship between fatty acid transporter expression and suppression of energy intake following intraduodenal lipid infusion in healthy humans*
Poster #21  Caroline Giezenaar, Laurence Trahair, Natalie Luscombe-Marsh, Rachael Rigda, Amy Hutchison, Christine Feinle-Bisset, Trygve Hausken, Karen Jones, Michael Horowitz, Ian Chapman, Stijn Soenen

The effects of protein on blood glucose concentrations and gastric emptying in healthy young and older subjects

Poster #22  Gabriella Heruc, Tanya Little, Michael Kohn, Sloane Madden, Simon Clarke, Michael Horowitz, Christine Feinle-Bisset

Attenuated postprandial blood glucose response and delayed gastric emptying are improved with refeeding in anorexia nervosa

Poster #23  Amy T. Hutchison, Christine Feinle-Bisset, Penelope C.E. Fitzgerald, Scott Standfield, Michael Horowitz, Peter M. Clifton, Natalie D. Luscombe-Marsh

Comparative effects of intraduodenal protein on gut motility, hormone release, glycemia, appetite and energy intake in lean and obese men

Poster #24  Amy Hutchison, Diana Piscitelli, Michael Horowitz, Karen Jones, Trygve Hausken, Christine Feinle-Bisset, Natalie D. Luscombe-Marsh

Load-dependent effects of oral protein on gastric emptying, glycaemia and energy intake in healthy men

Poster #25  Robert E. Steinert, Maria Landrock, Sina S. Ullrich, Scott Standfield, Bärbel Otto, Michael Horowitz, Christine Feinle-Bisset

Effects of intraduodenal L-leucine on antropyloroduodenal motility, gut hormone, insulin and glucagon release, blood glucose and energy intake in healthy men

Poster #26  Laura E. Claridge, Neha V. Valiyapurayil, Nam Q. Nguyen, Christopher K. Rayner, Tongzhi Wu, Jenna E. Burgess, Nektaria Pezos, Richard L. Young

Regional variation in expression of enzymes that synthesise incretin hormones and glucagon in lean and morbidly obese humans

**Session:**  Motility – animal models

Poster #27  Kyra J. Barnes, Nick J. Spencer

Do colonic migrating motor complexes (CMMCs) occur in mice lacking the EDN3 gene?

Poster #28  Marcello Costa, Kelsi Dodds, Lukasz Wiklendt, Phil Dinning, John Arkwright, Simon J.H. Brookes, Nick J. Spencer

Spatio-temporal analysis of neurogenic states during propulsive motor activity in guinea-pig small intestine, distal colon and rat colon

Poster #29  Julie E. Dalziel, Wayne Young, Premysyl Bercik, Nick J. Spencer, Leigh J. Ryan, Kelly E. Dunstan, Stacey E. Burton, Jason S. Peters, Catherine M. Lloyd-West, Nicole C. Roy

Tracking gastrointestinal transit in pharmacological models of chronic dysmotility in aged rats
Poster #30  Kiyotada Naitou, Takahiko Shiina, John B. Furness, Yasutake Shimizu

*Intrathecal application of noradrenaline causes propulsive contractions of the colorectum in anaesthetized rats*

Poster #31  Ruslan V. Pustovit, Leni R. Rivera, Kiyotada Naitou and John B. Furness

*Investigation of the physiological roles of ghrelin receptors in control of defecation*

Poster #32  Laura Ellett, Lin Wai Hung, Rachel Munckton, Alexandra Grubman, John B. Furness, Anthony White, David I. Finkelstein, Kevin J. Barnham, Victoria A. Lawson

*Restoration of gastrointestinal function in MPTP model of Parkinson’s Disease*

Poster #33  Andre Y.F. Tan, Magdy Sourial, John M. Hutson, Bridget. R. Southwell

*Non-invasive measurement of gastric emptying and oral-rectal transit in young piglets*

Poster #34  Linda J. Fothergill, Leni R. Rivera, Brid Callaghan, Hyun-Jung Cho, David M. Bravo, Daniel P. Poole, TinaMarie Lieu and John B. Furness

*Effects of TRPA1 agonists on short circuit current in the mouse duodenum and colon*

**Session: Sensory – mechanisms**

Poster #35  Dale F. Sharrad, Timothy J. Hibberd, Melinda A. Kyloh, Simon J.H. Brookes, Nick J. Spencer

*Quantitative immunohistochemical co-localization of TRPV1 and CGRP in varicose axons of the murine oesophagus, stomach and colorectum*

Poster #36  Adam G. Humenick, Bao Nan Chen, Phil G. Dinning, Lukasz Wiklendt, Nick J. Spencer, Vladimir P. Zagorodnyuk, Marcello Costa, Simon J.H. Brookes

*What is the mechanical "adequate stimulus" for gut mechano-nociceptors associated with intestinal blood vessels?*

Poster #37  Melinda A. Kyloh, Nick J. Spencer

*Identification of different types of spinal afferent nerve endings that encode noxious and innocuous stimuli in the stomach and esophagus using a novel anterograde tracing technique*


*Chronic activation of the GC-C/cGMP pathway by linaclotide inhibits ascending nociceptive pathways and restores aberrant spinal cord signaling*

Poster #39  Joel Castro, Andrea M. Harrington, Stuart M. Brierley

*TRPV3 contributes to chronic visceral mechanical hypersensitivity.*

Poster #40  Joel Castro, Grigori Y. Rychkov, Andrea Ghetti, Andrea M. Harrington, Caroline Kurtz, Ada Silos-Santiago, Stuart M. Brierley

*Extracellular cGMP, the downstream mediator released in response to linaclotide-induced activation of guanylate cyclase- C (GC-C), reduces excitability of murine and human dorsal root ganglion neurons*
Poster #41  Bao Nan Chen, Adam G. Humenick, Phil G. Dinning, Lukasz Wiklendt, Nick J. Spencer, Marcello Costa, Simon J.H. Brookes

*Mechanical activation of spinal afferents from flat sheets of guinea pig distal colon*

Poster #42  Timothy J. Hibberd, Melinda A. Kyloh, Simon J.H. Brookes, David A. Wattchow, Nick J. Spencer

*Development of a novel preparation based on a transgenic CGRPa reporter mouse to directly correlate functional properties of colorectal primary afferent neurons with their neurochemical phenotype*

Poster #43  Leni R. Rivera, Ruslan V. Pustovit, Linda J. Fothergill, Christopher Leung, Peter Angus, John B. Furness

*Mucosal permeability changes following diets high in fat and advanced glycation end products*


*GC-C agonists as regulators of visceral sensation: modulation of mucosal sensitivity via the epithelial GC-C/cGMP pathway*

**Session:** Entero-endocrine

Poster #45  Richard L. Young, Christopher K. Rayner, Nam Q. Nguyen, Sony S. Thazhath, Tongzhi Wu, Jenna E. Burgess, Amanda L. Lumsden, Ravinarayan Raghupathi, Nektaria Pezos, Damien J. Keating

*Increased capacity for intestinal serotonin release in obese and diabetic humans*

Poster #46  Alyce M. Martin, Damien J. Keating

*Nutrient sensing by enterochromaffin cells in the gastrointestinal tract*

Poster #47  Kate E. Polglaze, Danqing Lin, Lu Liu, Anna K. Walduck, Paul P. Bertrand

*Helicobacter pylori infection increases 5-hydroxytryptamine release in mouse stomach*
WHAT HAS SENSORY BIOLOGY TOLD US ABOUT GUT DISEASE?

David Grundy

Department of Biomedical Science, The University of Sheffield, Sheffield S10 2TN, UK.

Gastrointestinal (GI) dysfunction can lead to generation of pain but is often associated with vague feelings of bloating, discomfort and nausea. A feature of functional GI disorders is hypersensitivity manifested as allodynia and hyperalgesia indicating that sensory signalling has become upregulated either in the periphery where afferent impulses are generated or in the CNS where sensory signals are interpreted, reflecting heterogeneity in underlying pathophysiology. Recent research into sensory signalling from the GI tract has lagged behind similar studies in somatic pain but nevertheless, it is apparent that there are shared underlying concepts involving inflammation and neuropathic drivers that maintain pain states long after the initiating insult has occurred. Moreover, the same sensory signals from the gut connect to reflexes that regulate motility, secretion, blood flow and immune function. Thus aberrant sensory signals can give rise to a constellation of sensory and GI motor consequences that are a hallmark of functional disorders. Recent studies of somatic and visceral pain have identified changes in ion channels and receptors that determine signal transduction and excitability in the peripheral endings. The local chemical environment also plays a role in maintaining aberrant signalling. This is apparent from mucosal biopsies taken from IBS patients where a variety of inflammatory mediators including 5-HT, histamine and proteases from mast cells contribute to increase sensory signalling and correlates with the degree of hypersensitivity. This presentation will review mechanisms that initiate and maintain chronic pain drawing on examples from both visceral and somatic literature. The mechanisms underlying neuroimmune modulation of GI signalling will be discussed in the context of diagnosis and treatment of the troublesome symptoms of IBS.
ANORECTAL SENSORY AND MOTOR DYSFUNCTION

Rebecca Burgell
Department of Gastroenterology, Alfred Hospital and Monash University, Melbourne, Victoria, Australia

In contrast to the sound understanding of the structural anatomy of the anorectum, the neuronal control of rectal function is less well understood. In simplistic terms, the role of the rectum and anus appears straightforward; to contain a gradually increasing faecal bolus so that when sufficient stool has been accumulated, it can be expelled at a socially convenient time. In reality, however, their roles are significantly more complicated. The regulatory control of the anorectum is complex, involving interaction between the extrinsic nervous system (spinal cord, brainstem, cerebral cortex), the intrinsic nervous system (enteric nervous system) and the somatic nervous system. Intact sensorimotor pathways, both rectal and anal, are essential to normal defaecation. Accordingly, disruption of normal anorectal neuronal function has the potential to contribute to significant defaecatory dysfunction. To this end, an overview of current understanding of anorectal sensorimotor control will be provided and the clinical endpoints associated with anorectal sensory and motor dysfunction will be explored.

RECENT PROGRESS IN LARGE BOWEL MOTILITY

Marcello Costa
Human Physiology and Centre for Neuroscience, FMST, School of Medicine, Flinders University, Bedford Park, SA 5042

Motility of the large intestine involves formation and propulsion of faeces. We combined methods to construct temporal maps of movements, intraluminal pressure, intraluminal flow to determine the motor patterns generated by different mechanical conditions in isolated segments of colon. We inferred the role of excitatory and inhibitory enteric pathways and demonstrated that polarized activation of enteric ascending excitatory and descending inhibitory reflex pathways propel the contents. In empty segments of colon spontaneous slowly propagating neural motor complexes (MMCs) are modified by the physical consistency of the contents to shape and propel the faeces. Increasing the surface area of boluses, lowering the viscosity of contents increase the speed of propulsion. At the colonic flexure faeces are formed into more solid pellets by a process that involves MMCs arriving from the proximal colon and a powerful underlying very slow myogenic pacemaker activity at the flexure. Using high resolution, EMCCD camera and loading a calcium indicator into multiple myenteric ganglia we visualized temporal activation of myenteric neurons along the colon, demonstrating for the first time that coordinated neuronal firing pattern is responsible for the MMCs. We are developing both in plastic and in silico models that encapsulate the proposed simplicity. Despite the diversity and apparent complexity of colonic movements, two fundamental neural mechanisms, namely a spontaneous, content independent slowly migrating motor activity coupled with a content dependent neuromechanical loop, shape and adapt propulsion to the physical characteristics of contents and interact with ongoing myogenic activity to form and propel faeces.
ENDOLUMENAL FUNCTIONAL LUMEN IMAGING PROBE (EndoFLIP) PROVIDES A VALID AND SENSITIVE DIRECT MEASUREMENT OF THE UPPER OESOPHAGEAL SPHINCTER COMPLIANCE IN PATIENTS WITH RADIOTHERAPY-RELATED PHARYNGEAL DYSPHAGIA

Peter I Wu1, Michal Szczesniak1, Julia Maclean1, Peter Graham2, Harry Quon3, Lennart K Choo1, Teng Zhang1, Ian J Cook1

Departments of Gastroenterology and Hepatology1, Department of Radiation Oncology2, and Department of Speech Pathology3, St. George Hospital, University of NSW; Departments of Radiation Oncology, Johns Hopkins Hospital, Baltimore, USA1

Background: Chemo-radiotherapy in the treatment of Head and Neck Cancer (HNC) with/without laryngectomy commonly causes dysphagia. Upper oesophageal sphincter (UOS) strictureing is an important contributor. The accuracy of indirect indicators of impaired UOS compliance, including manometry and radiography remains unclear. A direct quantitative technique of UOS compliance is needed. Hypotheses: i) UOS compliance is reduced in dysphagic HNC patients; ii) EndoFLIP is valid and sensitive in quantitation of UOS compliance and its alteration by endoscopic dilatation. Methods: We prospectively studied 16 patients with dysphagia following radiotherapy for HNC and compared them with 16 asymptomatic healthy controls. EndoFLIP calculates 16 cross-sectional areas (CSA) across an 8-cm fluid-filled bag and its corresponding intra-bag pressure during distension. The EndoFLIP measurements of the UOS were performed under sedation before and after endoscopic dilatation. Results: 2 of 16 (12.5%) HNC patients vs. 14 of 16 (87.5%) controls reached the maximal distension volume of 50ml (p=0.005). At the distension volume of 40ml, the mean CSA was lower in the HNC group vs. controls (95mm² vs. 191mm², p<0.001). Distensibility (CSA/mmHg) at the same distension volume was lower in HNC group vs. controls (2.29mm²/mmHg vs. 7.05mm²/mmHg, p=0.003). Of the 15 patients who underwent endoscopic dilatation, 13 patients (86.7%) had increased CSA, with a mean increment of 35.6mm² (95%CI [11.7-59.5]), p=0.007)(Figure 1). Conclusions: 1) Radiotherapy-related dysphagia is associated with poor UOS compliance, which is partly reversible by endoscopic dilatation. 2) EndoFLIP is a minimally-invasive, valid and sensitive method of quantifying UOS compliance, which may help define treatment efficacy and safety targets.

Figure 1. UOS distensibility in HNC group and controls

 Oral 10:00am

 Oral 10:15am

QUANTIFYING PHASE II PATERN OF THE MIGRATING MOTOR COMPLEX IN THE HUMAN SMALL BOWEL USING HIGH RESOLUTION FIBRE OPTIC MANOMETRY

John W. Arkwright1, Eveline Deloose2, Maura Corsetti2, Lukasz Wiklund3, Jan Tack3, Phil G. Dinning4, Nathalie Rommel3

1CSEM, Flinders University, Bedford Park, SA, Australia. 2TARGID, KULeuven, Leuven, Belgium. 3Human Physiology, Flinders University, Bedford Park, SA, Australia. 4Flinders Medical Centre, Bedford Park, SA, Australia.

Phase II of the migrating motor complex (MMC) is commonly referred to as random phasic activity. In this study we apply high resolution fiber optic manometry to study the phase II activity. A fiber optic manometry catheter (diameter 3 mm, 72 sensors at 10 mm spacing) was placed transnasally in 10 healthy female volunteers (37±4 years). The studies continued until two phase III MMC contractions occurred or for a maximum of 5 hours. In all subjects, propagating activity was identified in the stomach and small bowel. During phase II of the MMC, antegrade propagating sequences were identified throughout the small bowel (93.1 ± 12.4 / hr), travelling at a speed of 36.5 ± 5.3 mm/s, and extending over 16.6 ± 1.3 cm. Retrograde propagating sequences occurred at 8.1 ± 6.3 / hr; (P = 0.003). Their speed of propagation (28.8 ± 9.2 / hr) was similar to antegrade events, however they extended over shorter lengths of the small bowel (8.6 ± 0.8 cm; Range 4 – 16 cm; P = 0.0001). Long antegrade propagating sequences were closely correlated with bursts of ∼3 cycle per minute activity in the stomach. These gastric and small bowel propagating events became more prevalent immediately prior to phase III of the MMC. The recorded data indicate that coordinated, regular propagating activity, temporally related to gastric 3 cycle per minute clustered motor patterns, forms the majority of small bowel phase II MMC activity and may require the concept of “random phasic activity” to be redefined.
OBESITY DISRUPTS 17ß-ESTRADIOL SIGNALLING IN GASTRIC VAGAL AFFERENTS

Stephen Kentish1,2, Claudine Frisby1,2, Gary Wittert1,2,3, Amanda Page1,2,3

1Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, University of Adelaide; 2South Australia Health and Medical Research Institute and 3Royal Adelaide Hospital. University of Adelaide

17ß-Estradiol (E2) reduces meal size and overall food intake in ovariectomized rats (Physiol Behav 2010;99:142). Activation of E2 receptors in the arcuate nucleus reduces food intake, but the E2 receptors, ERα, ERβ and GPR30 are also present in the nodose ganglia (NDG). E2 has been shown to sensitize cultured vagal neurons (Am J Physiol 2009;297:C654) suggesting a peripheral site of action on food intake because activation of mechanosensitive gastric vagal afferents (GVA) induces satiety. The effect of E2 on GVA mechanosensitivity under normal dietary conditions and high fat diet induced obesity (HF-DIO) is unknown and we aimed to determine this. Mice (8wks old) were fed either a standard (SLD) or a high fat diet for 12wks (N=8/group). Single fibre recordings of GVA mechanoreceptors were made (J Physiol 2012;590:209) before and after exposure to E2 (0.01-1 nM). NDG ERα, ERβ and GPR30 mRNA levels were quantified by QRT-PCR. In SLD mice, E2 potentiated mucosal and tension receptor responses to mucosal stroking (10-1000mg, p<0.001) and stretch respectively (1-5g, p<0.05). In HF-DIO, E2 had no effect on mechanosensitivity of tension receptors, but inhibited responses of mucosal receptors (p<0.001). ERα, ERβ and GPR30 were all present in NDG with ERα mRNA 46 and 15 times more abundant than ERβ and GPR30 respectively (p<0.001). There was no difference in ERα, ERβ or GPR30 mRNA levels between SLD and HF-DIO mice. In conclusion, the effect of E2 on GVA changes from a potentiation to an inhibitory effect in HF-DIO, which could lead to reduced vagal satiety signalling.

MODULATORY EFFECT OF APELIN ON GASTRIC VAGAL AFFERENTS MECHANOSensitivity IN DIFFERENT FEEDING STATUS

Hui Li1,2, Stephen Kentish1,2, Claudine Frisby1,2, Gary Wittert1,2,3, Amanda Page1,2,3

1Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, University of Adelaide; 2South Australia Health and Medical Research Institute and 3Royal Adelaide Hospital.

Apelin is the endogenous ligand for the G-protein coupled receptor, APJ (Biochem J Phys Res Commun 1998;251:471-6). It is suggested that apelin has a central role in the control of food intake. [J Neuroendocrinol 2008;20:79-84]. Apelin is expressed in the gastric mucosa (Regul Pept 2005;129:37-41) and may therefore have a modulatory effect on gastric vagal afferents (GVAs). We aim to determine: 1) the effect of apelin on GVA mechanosensitivity in mice fed a standard laboratory diet (SLD), a high-fat diet (HFD) or were food restricted, 2) whether APJ receptors are expressed in GVAs and 3) the anatomical location of apelin in the gastric mucosa in relation to APJ positive cells. APJ mRNA levels in the GVAs were quantified using RT-PCR. Immunohistochemistry was used to determine the relationship between apelin and APJ in the stomach wall. Apelin reduced the mechanosensitivity of tension receptors in SLD fed and fasted but not HFD mice. Apelin had no effect on the mechanosensitivity of mucosal GVAs in SLD and HFD mice. However, after an overnight fast, apelin reduced the response of mucosal receptors to mechanical stimulation. APJ mRNA was undetectable in gastric GVA neurons. In the gastric mucosa, APJ-expressing cells were found adjacent to apelin-expressing cells. These data suggest the effect of apelin on GVAs is dynamic and related to the feeding state. Apelin may not have a direct effect on GVAs, but instead may have an indirect pathway via action on APJ expressing cells in the gastric mucosa.
Chronic abdominal pain is a common symptom of Inflammatory Bowel Disease (IBD) that often persists in the absence of active inflammation. While the mechanisms responsible for pain are unknown, preclinical evidence suggests plasticity within the spinal cord dorsal horn (DH) is involved. We therefore developed an in vivo preparation to study the properties of DH neurons that receive inputs from the colon. Mice (C57Bl/6j, male, ~P35) were anaesthetised (isoflurane, 1-3%) and the L6-S1 spinal cord segments were exposed. Whole-cell patch-clamp recordings were made from laminae I-II DH neurons. We tested whether neurons received colonic inputs via noxious colorectal distension (CRD, 80 mmHg) and then assessed neuron intrinsic and synaptic properties. Recordings were obtained from 80 DH neurons, 10 of which responded to noxious CRD. Action potential discharge was observed in response to CRD in 3/10 neurons, while the remaining 7 neurons responded with subthreshold depolarisation or hyperpolarisation. Most CRD-responsive neurons (80%) also had a cutaneous receptive field compared with <50% of CRD-nonresponsive neurons. Several differences were observed in the intrinsic and synaptic properties of CRD-responsive and -nonresponsive neurons. CRD-responsive neurons had a hyperpolarised resting membrane potential, larger rheobase currents, and reduced levels of excitatory drive. In summary, we have identified differences in the properties of neurons that receive visceral input in naïve mice. Our preparation, which allows in vivo patch-clamp recording, will permit future detailed analysis of the mechanisms that determine DH neuron excitability in inflammatory conditions that produce abdominal pain.

IN VIVO CHARACTERISATION OF SPINAL CORD NEURONS WITH VISCERAL INPUTS

Kristen E. Farrell, Michelle M. Rank, Simon Keely, Brett A. Graham, Robert J. Callister

School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW and Hunter Medical Research Institute, New Lambton Heights, NSW

INTRODUCTION: Dysphagia is a common complication of laryngectomy. The underlying mechanisms are incompletely understood. Both pharyngeal weakness and upper oesophageal sphincter (UOS) restriction are candidate mechanisms. AIM: To quantify pharyngeal and UOS biomechanics in laryngectomies and the changes with UOS dilatation using high-resolution manometry (HRM). HYPOTHESES: 1. Laryngectomies have reduced hypopharyngeal peak pressure (hPP) and increased UOS resistance. 2. UOS dilatation improves dysphagia and this improvement correlates with reduction in resistance across UOS. METHODS: We assessed deglutitive function using the Sydney Swallow Questionnaire (SSQ) and HRM in 27 total laryngectomies. The HRM catheter (Unisensor solid state, 25 sensors, 1 cm apart) was positioned across the pharynx and UOS. Concurrent pressure and videoradiography were recorded during 3x5ml barium-bolus swallows. hPP was defined as the average peak contractile pressures in the distal 3cm of hypopharynx. Hypopharyngeal intrabolus pressure (hIBP), a measure of UOS resistance was the pressure within the intrabolus domain recorded 1cm above the upper margin of the UOS at the mid-flow time point at that recording site. RESULTS: hPP was significantly lower in the laryngectomies compared to age-matched controls (114±41±4.9 vs 170±15.4mmHg, p=0.015), while hIBP was higher (26.7±4.1 vs 6±4.9mmHg, p=0.005). SSQ score was significantly higher in laryngectomies (70±7.80 vs controls 92±14mmHg, p=0.0001). In all 5 patients undergoing dilatation, hIBP fell significantly (Figure 1). This decrement was mirrored by symptomatic improvement. CONCLUSIONS: Reduced hPP and increased UOS resistance contribute to dysphagia in laryngectomies. The evidence for high UOS resistance is stronger as it is partly reversible by dilatation and commensurate with symptomatic improvement.

Figure 1: SSQ and IBP pre and post dilatation (*p<0.04, **p<0.01):

Oral 11:36am

BIOMECHANICS OF DEGLUTITIVE DYSFUNCTION FOLLOWING TOTAL LARYNGECTOMY

Teng Zhang, Michal Szczesniak, Taher Omari, Julia Maclean, Peter I Wu, Ian J Cook

1Dept. Gastroenterology & Hepatology & 2Dept. Speech Pathology, St George Hospital, Sydney; 3School of Medicine University of New South Wales, Sydney; 4School of Medicine Flinders University, Adelaide; Australia

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INTRODUCTION: Dysphagia is a common complication of laryngectomy. The underlying mechanisms are incompletely understood. Both pharyngeal weakness and upper oesophageal sphincter (UOS) restriction are candidate mechanisms. AIM: To quantify pharyngeal and UOS biomechanics in laryngectomies and the changes with UOS dilatation using high-resolution manometry (HRM). HYPOTHESES: 1. Laryngectomies have reduced hypopharyngeal peak pressure (hPP) and increased UOS resistance. 2. UOS dilatation improves dysphagia and this improvement correlates with reduction in resistance across UOS. METHODS: We assessed deglutitive function using the Sydney Swallow Questionnaire (SSQ) and HRM in 27 total laryngectomies. The HRM catheter (Unisensor solid state, 25 sensors, 1 cm apart) was positioned across the pharynx and UOS. Concurrent pressure and videoradiography were recorded during 3x5ml barium-bolus swallows. hPP was defined as the average peak contractile pressures in the distal 3cm of hypopharynx. Hypopharyngeal intrabolus pressure (hIBP), a measure of UOS resistance was the pressure within the intrabolus domain recorded 1cm above the upper margin of the UOS at the mid-flow time point at that recording site. RESULTS: hPP was significantly lower in the laryngectomies compared to age-matched controls (114±41±4.9 vs 170±15.4mmHg, p=0.015), while hIBP was higher (26.7±4.1 vs 6±4.9mmHg, p=0.005). SSQ score was significantly higher in laryngectomies (70±7.80 vs controls 92±14mmHg, p=0.0001). In all 5 patients undergoing dilatation, hIBP fell significantly (Figure 1). This decrement was mirrored by symptomatic improvement. CONCLUSIONS: Reduced hPP and increased UOS resistance contribute to dysphagia in laryngectomies. The evidence for high UOS resistance is stronger as it is partly reversible by dilatation and commensurate with symptomatic improvement.

Figure 1: SSQ and IBP pre and post dilatation (*p<0.04, **p<0.01):
CIGUATOXIN POTENTLY ACTIVATES COLONIC NOCICEPTIVE PATHWAYS WITH GREATEST EFFICACY IN A MODEL OF CHRONIC VISCERAL HYPERSENSITIVITY

Luke Grundy1,2, Joel Castro1,2, Ashlee Caldwell1,2, Sonia Garcia-Caraballo1,2, Jessica Maddern1,2, Andrea M. Harrington1,2, Grigori Y. Rychkov2, Richard J. Lewis3, Irina Vetter3, Stuart M. Brierley1,2

Visceral Pain Group1, Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, The University of Adelaide, SAHMRI, Australia2. Institute for Molecular Bioscience, The University of Queensland, St Lucia, Queensland 4072, Australia3.

Pain is detected by primary sensory afferents that project from peripheral tissues to the dorsal horn of the spinal cord, via activation of ion channels, including voltage gated sodium (Na+) channels. Ciguatoxin (CTX) is a potent Na+ channel activator, which upon ingestion of contaminated fish causes intense abdominal pain in humans. As such we used CTX to investigate the neural mechanisms and the Na+ channels underlying visceral pain. Using mRNA expression studies we showed a selective co-expression of multiple Na+ channels in colonic DRG neurons, and an up-regulation of specific tetrodotoxin (TTX) sensitive Na+ channels in a model of chronic visceral hypersensitivity (CVH). In ex-vivo colonic afferent studies, CTX potently activated 72% of colonic nociceptors as well activating a population of ‘silent nociceptors’. Correspondingly, in vivo intra colonic administration of CTX caused significant activation of nociceptive signalling pathways within the dorsal horn of the thoracolumbar and lumbosacral spinal cord, as well as altering spontaneous behavioural responses. Calcium imaging studies showed a significant increase in the number of CVH colonic DRG neurons responding to CTX (100%) compared with healthy controls. In CVH colonic DRG neurons a larger proportion of the CTX response was sensitive to TTX, as indicated by reduced CTX responses and fewer CVH colonic DRG neurons responding to CTX. Overall, these results suggest CTX is a novel tool for the investigation of the mechanisms underlying visceral pain and indicates a significant role for TTX sensitive Na+ channels in neuronal excitability. These results also suggest that Na+ channel up-regulation and activation is an essential component of the hyperalgesia and allodynia associated with CVH.
VIDEO-HRIM DIAGNOSIS OF PHARYNGEAL DYSFUNCTION
Michal M. Szczesniak
Department of Gastroenterology and Hepatology, University of New South Wales and St George Hospital, Sydney, NSW

Oral 2:40pm

Oropharyngeal dysphagia is common following stroke, in neurological disorders and after head and neck cancer treatment. In addition to reduced quality of life, patients with dysphagia are at risk of aspiration pneumonia, nutritional failure and death. In order to direct therapy, clinician assessing patients presenting with pharyngeal dysphagia needs to be able to reliably identify: 1) the presence and timing of aspiration as it can be ‘silent’ and missed during clinical assessment; 2) structural abnormalities such as strictures; 3) abnormal or absent motor patterns that transport food bolus through the pharynx and protect the airways.

Videofluoroscopy is currently the gold standard in assessing the pharyngeal swallow. While it reliably detects aspiration (and gross structural abnormalities), it has several shortfalls. It is largely subjective and provides at best semi-quantitative evaluation of the swallow. It is prone to operator bias and inter-observer variability. It cannot provide information about biomechanics of a swallow, such as propulsive and resistive forces, pressures and flow patterns.

Videofluoroscopy can be combined with pressure/impedance to provide biomechanical information but at the price of increased complexity, cost, time and expertise required to conduct such studies. Semi-automated analysis method such as automated impedance/manometry (AIM) removes the subjectivity and bias from analysis. Even in the absence of videofluoroscopy, impedance/manometry in a clinic setting can predict residue and aspiration, however many more questions need to be addressed before its true clinical utility can be appreciated. Is it reliable in all dysphagia populations? Can it predict clinically relevant outcomes such as aspiration pneumonia and death? Is it sensitive to changes following therapy or in tracking recovery or decline in target populations? This presentation will attempt to put these strengths, limitations and future directions into perspective.

THE CHICAGO CLASSIFICATION OF OESOPHAGEAL MOTILITY VERSION 3.0
Richard H Holloway
Department of Gastroenterology and Hepatology, Royal Adelaide Hospital

Oral 3:00pm

The Chicago Classification of oesophageal motility disorders was proposed in 2008 as a response to the development of high-resolution manometry (HRM) and topographic plots which could not be readily analysed using conventional approaches. A new set of measurement variables was created, based in principle from previous conventional analysis but specifically tailored for HRM. This classification uses a hierarchical approach that involves systematic evaluation and sequential prioritization of lower oesophageal sphincter relaxation and major and minor disorders of peristalsis.

The classification is a work in progress and has undergone revision, the latest published in early 2015. The major changes that have occurred with the latest iteration are: (i) use of a median cutoff IRP value amongst swallows to define lower oesophageal sphincter relaxation, (ii) use of the distal latency rather than contractile front velocity to diagnose diffuse oesophageal spasm, (iii) incorporation of the lower oesophageal sphincter into the measurement domain for the distal contractile integral, (iv) basing weak peristalsis on DCI, simplification and rationalisation of disorders and abnormalities of peristalsis. The definition “hypercontractile oesophagus” has become slightly more restrictive, but has also included hypercontractility confined to the LOS segment. Revisions to the classification of peristaltic abnormalities include: renaming as “minor disorders of peristalsis”, disregard for small peristaltic breaks, creation of a new category, “fragmented” peristalsis, and re-inclusion of “ineffective” peristalsis.

The revisions have resulted in a classification that is better matched to clinical manometric experience.
EXTRINSIC SENSORY NEURONS, THE INTERFACE BETWEEN THE GUT AND THE BRAIN

David Grundy

Department of Biomedical Science, The University of Sheffield, Sheffield S10 2TN, UK.

The gastrointestinal tract has a rich sensory innovation yet sensory experiences are vague and poorly defined. Afferent impulses are conveyed along vagal and spinal pathways to the brain stem and spinal nuclei where signals are disseminated to various higher centres involved in mediating sensation, behavioural responses to luminal nutrients and reflexes involved in regulating a variety of GI functions. Distinct populations of afferent endings have been identified, terminating in different regions of the GI tract and in different layers of the gut wall. Those in the muscle, serosa and mesenteric attachments are mechanosensitive detecting distention and distortion of the gut wall. Other endings below to the mucosal epithelium and in the sub-mucosal layer are chemosensitive, responding to a diversity of mediators released from various cells involved with mucosal homeostasis and immune function. One such mediator is ATP that acts on purinoreceptors expressed on different elements of the gut wall. A direct effect of ATP on extrinsic sensory neurons is implicated in mechanotransduction and nociception. There may also be an indirect role for ATP via P2X7 receptors (P2X7Rs) located on immune cells. Inflammation can trigger persistent hypersensitivity to distention. This hypersensitive state arises because of altered excitability of the sensory endings following phenotypic changes in ion channels on the nerve ending or changes in the chemical milieu that influence sensory signal transduction via ligand gated ion channels. Hypersensitivity is absent in postinfected P2X7R−/− animals implicating ATP in intestinal inflammation and an early trigger for the development of chronic visceral hypersensitivity.

INTRINSIC SENSORY NEURONS, NOT JUST PRIMARY AFFERENTS

Joel C Bornstein, Rachel M Gwynne, Katerina Koussoulas, Jaime PP Foong

Department of Physiology, School of Biomedical Sciences, University of Melbourne

Some neurons in the intestinal wall act to detect the state of the intestinal lumen and muscle coat and regulate enteric neural circuit activity that controls functions like motility and electrolyte transport. These neurons have been identified electrophysiologically and morphologically as AH/Dogiel type II neurons and are known as intrinsic sensory neurons (ISNs) or intrinsic primary afferent neurons (IPANs). While some functions of these neurons are clear as they respond to chemical stimuli (amino acids, short chain fatty acids, acid pH, serotonin, ATP) applied to the mucosa, to mucosal deformation and to local distortion of the myenteric plexus, others remain obscure. This is partly because these neurons receive slow excitatory synaptic inputs from nearby AH/Dogiel type II neurons and descending interneurons and partly because the stimuli used to characterise them are typically too brief to reflect the physiological reality that changes in the properties of the gut are usually slow. AH/Dogiel type II neurons form recurrent excitatory networks and computer simulations exploring the implications of this reveal that this is important for their responses to sustained variable stimuli. This circuitry also means that they can amplify the effects of a local stimulus, even when they do not directly detect it, i.e. they act as interneurons under some conditions. Interestingly, pathogenic stimuli such as inflammation, cholera toxin and Clostridium difficile toxin produce long term increases in their excitability, thereby increasing the gain of enteric neural circuits making them more responsive to innocuous stimuli and enhancing their interneuron functions.
HIGH FAT DIET-INDUCED OBESITY DISRUPTS CIRCADIAN VARIATIONS IN GASTRIC VAGAL AFFERENT SATIETY SIGNALS IN MICE

Stephen Kentish1,2, Stamatiki Kritas2, Gary Wittert1,3,4, David Kennaway2, Amanda Page1,3,4

1Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine & 2Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, 3South Australia Health and Medical Research Institute, 4Royal Adelaide Hospital and 5Women’s & Children’s Hospital, Adelaide, South Australia

Mechanosensitive gastric vagal afferents (GVAs) are part of a co-ordinated set of peripheral mechanisms involved in the regulation of food intake. Previously we have shown that GVA response to mechanical stimulation exhibits circadian variation (J. Neuro 2013;33:19238-42) and that obesity reduces GVA mechanosensitivity (J. Physiol 2012;590:209-21). However, the effect of obesity on circadian variation of GVA activity is unknown and thus we aimed to determine this. Mice were fed either a standard laboratory diet (SLD) or high fat diet (HFD) for 12wks then sacrificed at 3hr intervals. Single fibre recordings from GVAs were obtained at each time point. C1 octanoid acid breath testing studies (Eur.J.Clin.Invest. 2002;32:341-4) were performed to determine the gastric emptying rate. In SLD mice, at 00:00 (N=6) as compared to 12:00 (N=6), stomach contents were 175% greater (p<0.001, one-way ANOVA, Tukey Posy-Hoc test), response of tension receptors to tension (3g) was reduced by 60% (p<0.001, one-way ANOVA, Tukey Posy-Hoc test) and that of mucosal receptors to stroking (50mg) was reduced by 60% (p<0.001, one-way ANOVA, Tukey Posy-Hoc test). In HFD mice circadian variation in stomach contents, vagal afferent mechanosensitivity was minimal. There was little variation in liquid gastric emptying in either SLD (1/2 28.2±1.1min at 18:00–38.8±5.2min at 00:00) or HFD mice (1/2 31.9±3.5min at 18:00–35.0±4.4min at 00:00) (both p>0.05 one-way ANOVA) and there was no difference between SLD and HFD mice (p>0.05, SLD vs. HFD two-way ANOVA, diet effect). In conclusion, HFD induced obesity suppresses circadian variations in gastric vagal afferent mechanosensitivity.

A VISCERAL REPRESENTATION OF ITCH: IDENTIFICATION ‘ITCH-SPECIFIC’ PRURITOGENIC MECHANISMS WITHIN COLONIC SENSORY PATHWAYS.

Joel Castro1, Andrea M. Harrington1, TinaMarie Lieu2, Sonia Garcia-Caraballo1, Jessica Madden1, Tracey O’Donnell1, Luke Grundy1, Daniel P. Poole1, Nigel W. Bunnett2, Stuart M. Brierley1

1Visceral Pain Group, Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, The University of Adelaide, SAHMRI, Australia. 2Monash Institute of Pharmaceutical Sciences, Monash University, Victoria, Australia.

Itch, like pain, is a major protective mechanism. Itch removes irritants from the skin, whilst pain allows withdrawal or avoidance of tissue damage. Painful and pruritogenic stimuli activate the peripheral endings of primary afferents that innervate second order neurons within the spinal cord. Whilst pain arises from both the skin and viscera; we investigated whether ‘itch-specific’ pruritogenic mechanisms also have functions within visceral pathways. Colonic DRG neurons express receptors for pruritogens, including TGR5 (15%), MrgrpA3 (33%) and MrgrpC11 (33%), in addition to GRP (20%), NPPB (20%), TRPA1 (43%) and TRPV1 (64%). Notably, expression of TGR5, MrgrpA3, and MrgrpC11 was also evident in the colonic mucosal epithelium. In ex vivo and in vivo functional studies TGR5 agonists excite colonic afferents and amplify responses to mechanical stimuli, consistent with neuronal sensitization. These effects were greater in colonic afferents from tgr5-tg mice, and lost in afferents from tgr5-tg and trpa1-tg mice. Chloroquine and BAM8-22 both excited colonic afferents and caused activation of dorsal hom neurons in the spinal cord. CVH mice showed a sustained amplification of the capacity of irritants to activate and sensitize colonic sensory neurons, suggesting functional up-regulation of this signalling pathway. Overall we have found that itch specific pruritogenic receptors are also expressed in colonic sensory pathways. In CVH mice colonic sensory pathways display increased responsiveness to known pruritogens. We propose that this colonic irritant sensing system is a visceral representation of the itch pathway in skin, and that it contributes to the sensory disturbances that accompany intestinal disorders, such as IBD and IBS.

Supported by NHMRC Australia
FAECAL MICROBIOTA TRANSPLANT IN ULCERATIVE COLITIS

Sam Costello
Gastroenterologist, Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville, South Australia.
IBD research fellow, Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, Adelaide, South Australia.

Ulcerative colitis (UC) is a chronic inflammatory bowel disease, that is characterized by continuous colonic inflammation extending proximally from the rectum. UC is associated with reduced luminal bacteria diversity and a mucosal inflammatory profile involving a complex mixture of innate and adaptive immune cells. Animal studies have led to a hypothesis that UC is characterised by a Natural Killer T-cell driven IL-13 and IL-5 dependent, TH2 mediated, immune response initiated by a loss of immune tolerance to colonic biota. There is little human data to validate this however. There is evidence that the microbiota are involved in the disease process as diversion of the faecal stream away from the colon can improve colonic inflammation and antibiotics have been shown to improve UC. Additionally many of the genetic risk alleles associated with UC are related to microbes and mucosal defense. Attempts have therefore been made to treat UC by manipulating the microbiota by faecal microbiota transplant with only anecdotal evidence of success at this point in time. There are randomized control trials currently underway to determine whether FMT is a clinically effective therapy. This research should also give an insight into interaction between the luminal microbiome and the mucosal immune system that occurs in active UC.

The FIRST-UC study is supported by the Gutsy foundation and the NHMRC

INFLAMMATION AND SENSORY FUNCTION

Patrick Hughes
Centre for Nutrition and Gastrointestinal Diseases, Dept. Medicine, University of Adelaide, AUSTRALIA

Inflammatory Bowel Diseases (IBD), incorporating Crohn’s disease and Ulcerative Colitis, are classic organic gastrointestinal diseases characterised by the presence of inflammatory lesions in the lower gastrointestinal tract. In contrast, Irritable Bowel Syndrome (IBS) is diagnosed by the presence of symptoms that occur in the absence of readily observable pathophysiological or biochemical abnormalities, and is classified as a functional gastrointestinal disorder. While disordered immune function is obviously implicated in IBD, there is increasing evidence that immune function is also altered in IBS patients. Further, IBD patients in remission frequently report symptoms of IBS leading to the controversial theory of IBD-IBS and indicating the underlying mechanisms of these diseases may potentially be shared. Animal models have provided important insights into the mechanisms underlying inflammation and how immune mediators modulate nerve function in naïve animals, but are rarely followed into remission or relapse. In contrast, the majority of human studies investigate relapsing, and not naïve, IBD. Much remains to be understood regarding which arms of the immune system are most important in IBS and IBD, the immune cell types and mediators involved and how these mediators alter gastrointestinal nerve function. These questions are difficult to answer without first characterising the types of immune responses that occur in IBD and IBS. Understanding how immune responses differ between IBD and IBS and how these differences lead to symptoms is likely to provide clues toward causation, biomarkers and novel leads for treatment.

PAH is supported by supported by NHMRC Australia.
MESENCHYMAL STEM CELL-BASED TREATMENTS FOR ENTERIC NEUROPATHY ASSOCIATED WITH COLITIS

Ainsley Robinson, Sarah Miller, Samy Sakkal, Kulmira Nurgali

College of Health and Biomedicine, Victoria University, Melbourne, Australia

Damage to the enteric nervous system (ENS) associated with intestinal inflammation underlies persistent alterations in gut functions controlled by the ENS, suggesting that enteric neurons are viable targets for novel therapies. Mesenchymal stem cells (MSCs) offer beneficial therapeutic effects for attenuation of neurodegenerative diseases by homing to areas of inflammation and exhibiting neuroprotective, anti-inflammatory, and immunomodulatory properties. MSC- released soluble bioactive factors promote neuronal survival and suppress inflammation suggesting that MSC-conditioned culture medium (CM) could provide all essential factors to repair damaged tissues. However, no studies evaluated the potential of MSCs and CM to attenuate enteric neuropathy associated with IBD. We have studied neuroprotective potential of MSC and CM treatments in two animal models, guinea-pigs with acute trinitrobenzene-sulfonate (TNBS)-induced colitis and Winnie mice with spontaneous chronic colitis. In Winnie mice the efficacy of MSCs administered via enema, intraperitoneal and intravenous routes have been compared. The effect of MSC and CM treatments was assessed by histological, immunohistochemical, and motility analyses at 6, 24, 72 hours and 7 days post-treatment. MSC and CM treatments prevented weight loss and gross morphological damage in the colon, decreased the quantity of immune infiltrate in the colonic wall and at the level of the myenteric ganglia, prevented neuronal loss and axonal damage, and alleviated inflammation-induced colonic dysmotility. MSCs and CM treatments were effective within 24 hours in animals with acute colitis and after 3 days in animals with chronic colitis.

EXPRESSION AND DISTRIBUTION OF THE DELTA OPIOID RECEPTOR IS ALTERED IN ACUTE COLITIS

Jesse J. Di Cello, Tina Marie Lieu, Emily M. Eriksson, Cameron J. Nowell, Meritxell Canals, Nigel W. Bunnell, Daniel P. Poole

1Monash Institute of Pharmaceutical Sciences, Parkville, VIC 3052, 2Walter & Eliza Hall Institute, Parkville, VIC 3052

Introduction. Opioids regulate intestinal motility and secretion and are major suppressors of inflammatory signaling. Although recent studies highlight the role of endogenous immune-derived enkephalins in inflammatory bowel disease, the relative contribution of the delta opioid receptor (DOR) to colitis development is presently unknown. We examined inflammation-associated changes in DOR distribution in the colon and assessed the contribution of DOR to proinflammatory signaling. Methods. Acute colitis was induced in DORGFP knockin mice and C57BL/6 mice (3% DSS, 5d). Disease activity and tissue damage were assessed. DOR distribution was determined in wholemount preparations by confocal microscopy and the subcellular distribution of DOR and innervation density were analyzed from captured images. Results. DORGFP was localized to the cell surface of a subset of myenteric neurons in control tissues. There was a significant increase in intracellular DOR and loss of cell surface-associated receptor in the acutely inflamed colon, indicative of endocytosis (P<0.001, n=12 mice). Internalization of DOR was attenuated following treatment with the DOR antagonist naltirindole (P<0.05). Colitis was associated with an increase in density of DOR-positive nerve fibers in the muscularis externa relative to controls. Naltrindole significantly increased relative weight loss and colon shortening (n=12, p=0.003), and histological damage scores (P<0.05). Conclusions. DOR contributes significantly to disease severity in an acute model of colitis. The endocytosis of DOR in inflamed tissues suggests a sustained exposure to endogenous DOR agonists in disease. The inhibition of DOR results in augmented disease activity and histological damage, consistent with a protective role for DOR in colitis.
IRRITABLE BOWEL SYNDROME (IBS): PHARMACOTHERAPY VERSUS DIET VERSUS PSYCHOLOGICAL TREATMENTS

Nicholas J. Talley

University of Newcastle, Australia

Treatment options are currently based on predominant symptoms, and treatment is symptom based rather than pathophysiologically driven, but this is arguably beginning to change. Therapies that work locally in the gut rather than systemically for IBS with constipation include linaclotide, a guanylate cyclase-C activator, and lubiprostone, a bicyclic fatty acid. Inflammation marks a subset with IBS, including mast cell infiltration in the small and large intestine. This is associated with increased gut permeability and mucosal immune activation with cytokine elevation that may drive the extra-intestinal manifestations of IBS (e.g., anxiety, fatigue). Steroids and 5-ASA have proven disappointing in randomised controlled trials. Mast cell stabilizers are not of proven benefit but antihistamine data look more promising. Excess small bowel bacteria have been documented in IBS by quantitative culture studies; abnormal colonic flora also occurs. Only one drug is known to temporarily alter the natural history of IBS (rifaximin, 10% therapeutic gain). Select probiotics may be beneficial but data remain contradictory. Decreasing or increasing bile acids may be beneficial in IBS. New data suggest early diet intervention early may be valuable (low FODMAP with up to 70% responding who can comply short term, or a gluten free diet in non-celiac patients). Diet restriction reduces bacterial substrate, reducing gas production and pain and bloating. Food intolerance may also alter gut function, or through allergic mechanisms induce symptoms in subsets. Notably a 2014 meta-analysis (ACG) ranked the evidence quality for specialised diet studies as very low. In patients failing first line therapies, antidepressants are superior to placebo in IBS. The long-term risks of antidepressants is currently controversial. Psychological therapies in the most recent (ACG) meta-analysis showed efficacy in IBS but the results are complicated by heterogeneity and risk of bias (evidence quality very low). The major barrier to use of these therapies is the lack of skilled therapists; internet delivery is of increasing interest.

DIET

Peter Gibson

Department of Gastroenterology, Alfred Hospital & Monash University, Melbourne

More than 60% of patients link triggering or aggravation of IBS symptoms to ingestion of specific foods. Patients use dietary manipulation to help control symptoms and, together with the community in general, have developed often intense interest in specialised, restrictive diets, such as those that target multiple food groups (e.g., paleo diet), avoid wheat or gluten, or reduce FODMAP intake. Gastroenterologists are now starting to take the initiative by the early use of dietary regimens in patients with IBS, based upon evolving evidence of the efficacy of dietary approaches. Most focus has been on FODMAPs; reducing their intake provides good symptomatic relief in about 70% of patients as reported in RCTs and observational studies internationally. Good supporting materials including booklets and an app assist with its implementation. Gluten or wheat-free diets have some evidence for efficacy in non-coeliacs, but it is not possible at present to readily define patients who are wheat-protein sensitive. Low bioactive chemical diets have a theoretical basis but no quality studies to guide their use. The risks of restrictive diets such as nutritional inadequacy, precipitation of eating disorders and adverse effects on gut microbiota must be considered although specific data on them and their health consequences are scant. In the spectrum of therapies for IBS, diet now sits for many patients as the first-line modality of attack, especially since increments and frequency of responses are far greater for the low FODMAP diet than for pharmacotherapy. All gastroenterologists should be on board!
THE ROLE OF PSYCHOLOGICAL THERAPIES IN FUNCTIONAL GI DISORDERS

Jim Kantidakis

The Gut Centre, Melbourne Victoria

Functional digestive disorders, such as IBS, have a considerable negative impact on a sufferer’s quality of life. Certain personality and psychological characteristics of IBS patients further perpetuate the symptoms and can worsen severity and distress. Psychological therapies, including Gut Directed Hypnotherapy and Cognitive Behavioural Therapy (CBT), have been found to reduce physical symptoms and psychological distress resulting in improved quality of life.
COMBINING EXISTING LAXATIVES TO DISIMPACT THE REALLY HARD CASES: PRODUCING EFFECTIVE COLONIC MOTILITY IN CHILDREN WITH CHRONIC CONSTIPATION AND PALPABLE FAECALOMA.

Lauren D. DuPège 1, 2, Julie Jordan-Ely 1, Kyla Dobson 1, Lefteris Statopoulos 1, 3, Marcelo Leaf 1, Tony Catto-Smith 1, 2, John M. Hutson 1, 2, 3, Bridget R. Southwell 1, 2, 4

1 Murdoch Childrens Research Institute, Parkville, Vic, Aus; 2 Department of Paediatrics, University of Melbourne, Parkville, Vic, Aus; 3 Department of Urology, Royal Childrens Hospital, Parkville, Vic, Aus; 4 Dept of Gastroenterology & Clinical Nutrition, Royal Childrens Hosp, Parkville, Vic, Aus.

Polyethylene glycol (PEG) is the gold standard oral laxative for faecal disimpaction and is commonly combined with a stimulant for greater effect. The stimulant laxative (sodium picosulphate, SPS) is well tolerated by children. **Aim:** Determine the effectiveness of combined PEG/SPS for increasing colonic motility to disimpact children with chronic constipation with a palpable faecaloma. **Methods:** 22 children (12 male, 4-17yrs) 2-3 yrs chronic constipation, palpable faecaloma and enlarged stool-filled rectum (rectal pelvic ratio >0.6) on x-ray. Daily diary recorded stool volume and laxative dose. Median laxative dose was 5.4, 2 sachets of Movicol (PEG + electrolytes, 14.7 g/sachet, 1 sachet/250ml water +125ml juice/milk) and 0.12, 15 drops of SPS on Day 1, 2, 3, then 1 sachet of Movicol plus 10 drops of SPS for 14 days. Children drank 250-500ml/hour using a fun approach (MOTIVATE). **Results:** Stool became soft without producing diarrhoea. Defecation frequency increased (median 6 to 18.5 bowel motions/wk). Stool volume (median) increased from 1.0L/wk pre to 2.3L/wk with maximal volume on day 2, 0.5-4.0L of stool over day 1-4 and ≥250ml/day for day 5-14, with 4.2±0.6L total stool (mean±SEM) over 14 days. The rectum was emptied in 1/3 of patients but remained distended. In the other 15/22, faecalomas were reduced in size. Children were easily able to drink the large volumes of PEG solution using the MOTIVATE method. **Conclusion:** High dose PEG/SPS produced large volumes of soft stool, completely removed hard faeces in 1/3 of children and reduced the size of stool mass in the other 2/3. A slightly larger dose is likely to empty the faecaloma in all children.

DISIMPACTION OF CHILDREN WITH CONSTIPATION IN A SUBURBAN CLINIC USING POLYETHYLENE GLYCOL AND SODIUM PICOSULPHATE.

Julie Jordan-Ely 1, John M. Hutson 1, 2, 3, Bridget R. Southwell 1, 2, 4

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Constipation can become chronic leading to faecal impaction. Protocols combining high dose osmotic laxatives with stimulants are used for bowel clearance prior to colonoscopy, but have not been reported for treating faecal impaction. **Aim:** Assess the effectiveness of a faecal protocol using high dose polyethylene glycol with electrolytes (PEG+E: Movicol, osmotic) combined with sodium picosulphate (SPS: Dulcolax SP drops, stimulant) on faecal impaction in children presenting to a suburban clinic. **Methods:** Forty-four children (aged 2-17 years) presenting to a suburban continence clinic over 8 months with constipation and faecal impaction were treated. Children took 6-8 sachets of PEG+E on day one, decreasing doses on days 2-3, and 15-20 drops SPS on days 2 and 3. On days 4-7, PEG+E reduced to 1 sachet and SPS to 10 drops. Defecation, soiling, diet and water intake was recorded in a daily diary for 7 days. **Results:** All children began defecating within 10-12 hours, producing (mean±SEM) 1±0.2, 4±0.3 and 3±0.3 cups of stool on days 1, 2 and 3 and then continued to produce 1±0.1 cup of stool/day. All achieved a feeling of complete emptying. Stool consistency changed from dry (Bristol Stool Scale, BSS3) on day 0, to loose (BSS5) 6 days 2&3 and back to formed (BSS4-5) for day 4-7. Despite soft stool, there was no faecal soiling, nausea, vomiting, or abdominal pain. **Conclusions:** A high-dose oral protocol combining PEG+E and SPS drops successfully removed residual hard faecal mass in children with acute/chronic constipation presenting to a suburban continence clinic.
DEVELOPMENT OF RHYTHMIC: A MEDICAL DEVICE TO INCREASE BOWEL MOTILITY AND TREAT CONSTIPATION.

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Over 10 years, we showed that transcutaneous electrical stimulation (TES) using interfential current can increase defecation to overcome constipation in children and adults. Others have shown the method is also helpful for functional dyspepsia and spina-bifida. Existing devices were good for physiotherapists but difficult for patients to use. We received funding to develop a prototype device specifically for treating constipation. **Aim:** To describe the progress in designing, testing and manufacturing the new device. **Results:** First we patented the method and then were successful to get a NHMRC Development Grant to develop the prototype. This funding allowed us to create a research device, begin animal studies and to apply for further investment. We received investment funding from the Medical Research Commercialisation Fund to further design and develop the prototype and take to market. We established a Start-up company - GI Therapies with a CEO experienced in developing medical devices. The product **Rhythm.IC,** has been designed by engineers and industrial designers with regulatory requirements covered by an experienced regulatory firm. **Rhythm.IC** is a battery-operated system used by the patient at home to deliver stimulation through 8 electrodes in two banks allowing activation of the transverse colon and rectum. Delayed gastric emptying can be treated by positioning the belt over the stomach. Leads are enclosed in a garment with simple connections ensuring the patient always receives the correct stimulation. The device logs usage providing clinicians with a clear record of compliance. Rhythm.IC is undergoing CE mark audit in March for release in Europe mid-2015.

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TRANSCUTANEOUS ELECTRICAL STIMULATION ACROSS THE ABDOMEN IMPROVES SYMPTOMS IN ADULTS WITH GASTROPARESIS: A PILOT STUDY

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Functional dyspepsia (FD), defined as upper abdominal pain/discomfort without organic disease, affects 5% of Australians. Symptoms include postprandial fullness, early satiety, epigastric pain with nausea, anxiety/depression. Gastroparesis (GP) has similar symptoms with delayed gastric emptying. For patients with treatment-resistant FD/GP treatment options are limited. Transcutaneous electrical stimulation (TES) is a physiotherapy method with painless current applied through the skin. Previous studies showed that TES improved FD. **Aim:** Determine if TES improves GP symptoms. **Method:** 6 adult females (25-40 yrs) with severe treatment-resistant GP (3 on total parenteral nutrition [TPN - nutrition via cannula into the veins]) and 1 on enteral feeding [nasogastric tube to stomach / jejunum]. All had constipation. Electrodes were placed on the abdomen and back over the diaphragm (T7-T10). Stimulation using interfential current was self-administered for 1 hr/day for 11-17 weeks. **Results:** All patients showed improvement: nausea, vomiting and bloating improved or stopped. Normal stomach and bowel actions developed in 4/6. 3/3 on TPN ceased TPN and resumed normal eating. One on enteral feeding resumed normal eating. 4/6 developed regular bowel actions, while 1/6 developed diarrhoea.

**Conclusion:** TES over the abdomen produces marked improvement in adult patients with severe GP.

Table 1: Effect of TES on GP symptoms:

<table>
<thead>
<tr>
<th>Age yrs</th>
<th>Stimulation</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>16wk</td>
<td>Off TPN, diarrhoea</td>
</tr>
<tr>
<td>29</td>
<td>14wk</td>
<td>Off TPN, regular bowel actions</td>
</tr>
<tr>
<td>27</td>
<td>12wk</td>
<td>Off TPN, bowel habit regular -daily/2nd daily</td>
</tr>
<tr>
<td>37</td>
<td>11wk</td>
<td>Off enteral feeding, bowel actions 2nd daily</td>
</tr>
<tr>
<td>29</td>
<td>17wk</td>
<td>Less bloating and nausea, bowel habit regular - off laxatives</td>
</tr>
<tr>
<td>25</td>
<td>11wk</td>
<td>Defecation most days, intermittent impaction</td>
</tr>
</tbody>
</table>
DISTINCT ALTERATIONS IN THE GUANYLATE CYCLASE-C/GMP PATHWAY ARE EVIDENT ACROSS DIFFERENT SUBTYPES OF IRRITABLE BOWEL SYNDROME PATIENTS

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Linaclotide, a guanylate cyclase C (GC-C) agonist, reduces abdominal pain and improves constipation in patients with Irritable Bowel Syndrome with Constipation (IBS-C). We have recently shown that linaclotide activates GC-C expressed on intestinal epithelial cells, resulting in the production and release of cyclic GMP (cGMP), which inhibits colonic nociceptors. We have shown that key components of the GC-C/cGMP signalling pathway are expressed within human colonic mucosa. However, it remains to be determined if components of this pathway are differentially expressed in different IBS patient subtypes. In mucosal biopsies from healthy controls, guanylin was the most abundantly expressed component of the GC-C/cGMP signalling pathway, followed sequentially by uroguanylin, GC-C, MRP5 and MRP4, respectively. In IBS-M biopsies both of the endogenous GC-C agonists, guanylin and uroguanylin, were significantly reduced compared with healthy controls. By contrast, in IBS-C patient biopsies, MRP4 was significantly down-regulated compared with expression in biopsies from healthy controls. No significant change in either MRP5 or GC-C expression was observed between IBS patient subtypes and healthy controls. Immunohistochemistry revealed MRP4 expression on the apical side of colonic epithelial cells, whilst MRP5 displayed basolateral expression. Distinct alterations in the GC-C/cGMP pathway are evident between different subtypes of IBS patients and may contribute to differences in bowel habit. In IBS-M reduced expression of the endogenous hormones guanylin and uroguanylin may contribute to alternating bowel habits. In IBS-C a reduction in apically expressed MRP4 may result in reduced release of cGMP into the colonic lumen. Overall, these changes may help to explain some aspects of the pathophysiology associated with IBS and the differential stool frequency and symptom patterns between IBS subtypes.

Supported by Ironwood Pharmaceuticals Inc. and NHMRC Australia.

PRESERVED COLONIC MEAL RESPONSE AND FUNCTIONAL EVIDENCE FOR ANASTOMOTIC NERVE REGENERATION IN PATIENTS WITH NORMAL BOWEL FUNCTION FOLLOWING ANTERIOR RESECTION

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Background: Preservation of normal bowel function after an anterior resection, suggests that normal colonic motor patterns are restored. Methods: In 15 patients (6 males; median age 68 years; 47-75 yr) that had undergone anterior resection >12 months prior, and reported normal bowel function, a fibre-optic, manometry catheter (36 sensors spaced at 1cm intervals) was positioned via colonoscopy into the distal colon with sensors straddling the site of anastomosis. Manometry was recorded 2hrs pre and post a 700cKal meal. These data were compared to 12 healthy controls (5 males; median age 51 yrs; 27-69yr). Results: Spectral analysis revealed a dominant pressure event frequency of 2-3cpm prior to the meal in the distal colon of both patients and healthy controls. In both groups the amplitude of the pressure events with this frequency increased significantly (P < 0.001) after the meal. In both patients and controls retrograde cyclic propagating motor patterns increased significantly after the meal (Patients; 2.1 ± 0.7 vs 32.6 ± 8.5 / 2hr; P < 0.001)(Health; 8.1 ± 3.8 vs 59.1 ± 25.7 / 2hr; P < 0.001). The delta increase did not differ between the groups (P = 0.3). Retrograde and antegrade propagating motor patterns were observed to cross the site of anastomosis in 11/15 patients. Conclusion: The restoration of normal distal colonic motility and propagation of motor patterns across the site of anastomosis suggests a degree of nerve regeneration in anterior resection patients with preserved normal bowel function.
UTILISING HIGH-RESOLUTION COLONIC MANOMETRY TO QUANTIFY DYSMOTILITY IN CHILDREN WITH SLOW TRANSIT CONSTIPATION.

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Background: Slow transit constipation (STC) is associated with colonic motor abnormalities in both adults and children. Methods: In 11 children (2 males; mean age 15.4 years; range 9-19 years) with STC, after an overnight fast, a 36 sensors (spaced at 1.5cm intervals) water perfused manometry catheter was placed and the tip clipped in the region of the splenic flexure. Manometry was recorded for two hours pre and post a 700cKal meal. These data were compared to 12 healthy adults (5 males; median age 51 years; range 27-76 years) with STC. Data in adults were recorded with a 72 sensors (spaced at 1cm intervals) fibre-optic manometry catheter.

Results: In healthy controls (HC) and adult STC patients, 2-3 cpm activity was prominent prior to and after a meal. This activity was not evident in children. In HC there was a significant postprandial increase in the count of retrograde cyclic motor patterns and this was not seen in either patient group (HC, 59.9 ± 25.4; STC adults, 4.9 ± 1.5; children, 2.4 ± 0.8 / 2 hr; ANOVA P < 0.0001). Conclusion: Neither patient group responded to a high calorie meal. The number of propagating events did not differ between STC adults and children, however in children the normal 2-3 cpm slow wave activity was not evident. The failed meal response and lack of prominent slow wave activity may indicate potential intrinsic and extrinsic abnormalities in children with severe constipation.

INTEGRATED RELAXATION PRESSURE (IRP4) INCREASES DURING VISCIOUS SWALLOWING IN AGED SUBJECTS

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1 Investigation and Procedures Unit, Repatriation General Hospital, Adelaide, South Australia; 2 School of Medicine, Flinders University, Adelaide, SA and 3 Women’s & Children’s Hospital, Adelaide, SA, Australia

Background: Recent manometric studies have shown decreased lower esophageal sphincter (LES) relaxation in both asymptomatic and dysphagic humans aged >80yrs especially during solid bolus swallows. The impact of this on LES clearance is unclear. The current study aimed to compare the effect of age on relaxation (IRP4) and clearance across the LES. Methods: Fifteen asymptomatic older healthy (85±4 yrs, 10M) and 15 young (27±7 yrs, 5M) participants underwent high-resolution impedance manometry (HRIM; MMS Solar GI System; Unisensor catheter; 25P 1cm spacing, 12 impedance 2cm segments). All subjects received five 5- and 10-ml liquid and viscous swallows in an upright posture. Data analyzed according to Chicago classification criteria, and automated impedance manometry (AIM) pressure flow analysis were compared for liquid and viscous swallows in the distal esophagus and LES. Clearance was assessed via standard criteria. Results: In older subjects, IRP4 was higher during viscous swallows compared to liquid (+4.2±1.8mmHg), in contrast to young subjects where the IRP was lower (-0.2±0.6mmHg; P<0.01, young vs. aged). The IRP4 increase was >3mmHg in 8 older, but only 1 young subject (P<0.01). Median IRP4 was above 15mmHg in 6 older subjects during viscous swallows. The increase in IRP4 with viscous did not alter LES bolus clearance. In subjects with an increased IRP4 relative to young controls, intrabolus pressure was increased (P<0.01) and nadir impedance was higher (P<0.01). Conclusion: Further studies are required to determine if high intrabolus pressure maintains bolus transit in aged subjects or is a causative factor in increased IRP4.
UPPER ESOPHAGEAL SPHINCTER (UES) MAXIMUM ADMITTANCE IDENTIFIES DYSPHAGIA DUE TO NEUROMUSCULAR AND STRUCTURAL PATHOLOGY

Charles Cook1,2, Stamatki Kritas3, Carly Burgstad1, Alison Thompson2, Laura Besanko1, Richard Heddle1, Robert J. Fraser1, Taher Omari1,2,3

1Investigation and Procedures Unit, Repatriation General Hospital, Adelaide, South Australia; 2School of Medicine, Flinders University, Adelaide, SA and 3Women’s & Children’s Hospital, Adelaide, SA, Australia

BACKGROUND: Upper esophageal sphincter (UES) nadir impedance has been shown to correlate with UES diameter on simultaneous radiology. This study compares the novel parameters of UES maximum admittance (inverse of impedance) with 0.2 sec integrated relaxation pressure (IRP). The aim was to identify neuromuscular and structural causes of dysphagia, and specifically manometric determination of UES opening. METHODS: Solid state high-resolution impedance manometry was performed in patients with criopharyngeal bar (CPB; n=11, aged 51-88yrs) or motor neuron disease (MND; n=13, 58-93yrs) and compared to 66 healthy subjects (21-9yrs). Data were acquired using a Unisensor catheter (25P121 or 32P160) during five 3ml liquid (L) and viscous (V) boluses. Statistical analyses included Kruskal Wallis one-way ANOVA and Mann-Whitney U-test on per subject mean values (IRP and UES maximum admittance). RESULTS: The IRP was significantly increased during liquid swallows in both healthy older subjects (aged >60yrs) and patients with MND (Figure; P<0.05), when compared to younger volunteers. In healthy controls, maximum UES admittance was lower in older age (>60yrs) for both liquid (P<0.001) and viscous swallows (P<0.05). Admittance was significantly reduced in CPB and MND patients when compared to young controls, during both liquid and viscous swallows (P<0.001). UES maximum admittance was also significantly lower when compared to age-matched controls in CPB (L,P<0.001; V,P<0.01) and MND (L,P<0.0001; V,P<0.01) (figure). CONCLUSION: UES maximum admittance clearly distinguished patients with both structural and neuromuscular causes of dysphagia from both young and older healthy subjects. IRP (0.2 sec) failed to distinguish dysphagic patients from healthy controls.

Poster #9

ENDORECTAL BALLOON (ERB) DURING IMAGE GUIDED RADIATION THERAPY (IGRT) FOR PROSTATE CARCINOMA (CAP) REDUCES RADIATION PROCTITIS AT 2 YEARS

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1Dept Radiation Oncology, Royal Adelaide Hospital, Adelaide, SA; 2Gastroenterology and Surgery, Flinders University, Flinders Medical Centre, Adelaide, SA

Radiation proctitis, characterised by symptoms such as increased frequency and urgency of defaecation, faecal incontinence and rectal bleeding frequently persist and have an adverse impact on quality of life of patients after IGRT for CaP. Daily insertion of a purpose-built ERB during IGRT reduces radiation exposure of the rectum and anal canal but hitherto, randomised trial evidence that GI symptoms are reduced have been limited to 1 month and 1 year. As the prevalence of radiation proctitis progresses with time, the findings at 2 years in 18 patients (74(55-84) yrs), 10 randomised to IGRT+ERB and 8 to IGRT alone are reported here. Each patient underwent evaluations of GI symptoms (LENT-SOMA questionnaire) and anorectal motor and sensory function (manometry with graded balloon distension) at baseline and at 1 month and 1, 2 and 3 years after completion of IGRT. Measures of GI symptoms and anorectal function were analysed with ANOVA. In the whole patient group, total GI symptom score (p<0.01), stool frequency (p<0.05) and rectal mucous discharge (p<0.05) increased from baseline 2 years after IGRT and were associated with progressive reductions in rectal compliance (p<0.05). There was a greater prevalence of rectal bleeding in patients who had IGRT alone compared to the IGRT+ERB patients at 2 years (p<0.05) but no other differences in GI symptoms and anorectal function parameters between the two patient groups. In conclusion, the prevalence of rectal bleeding 2 years after IGRT for prostate carcinoma is reduced by utilising ERB during treatment.
ENTERIC NEUROPATHY INDUCED BY ANTI-CANCER CHEMOTHERAPEUTIC DRUG OXALIPLATIN

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Colorectal cancer (CRC) is a leading cause of death worldwide. Chemotherapy alone, or in combination with radiation, is given before or after surgery to most CRC patients. Although chemotherapeutic drugs increase survival rate, they have severe gastrointestinal side-effects such as diarrhoea, constipation, oral mucositis, nausea and vomiting. These side-effects might last up to 10 years after cessation of the treatment. The traditional view is that gastrointestinal side-effects of anti-cancer drugs are due to mucosal damage. Neurotoxic effects of anti-cancer drugs on the intestinal innervation have not been studied in depth and may contribute to the side-effects of chemotherapy. In this study we have investigated the effects of chronic intraperitoneal administration of oxaliplatin, one of the first-line therapies to treat colorectal cancer, inducing peripheral neurotoxicity and severe gastrointestinal side-effects. Oxaliplatin (30 mg/kg/d) was administered in vivo to Balb/c mice intraperitoneally three times a week. Animals displayed symptoms of watery diarrhoea and the early stages, pica and constipation at the late stages of the treatment. Colonic dysmotility observed after long-term in vivo oxaliplatin treatment was associated with increases in neuronal nitric oxide (NO) synthase, enhancement in NO-induced colon constriction studied in organ-bath and force transduction experiments as well as NO-induced neuromuscular transmission studied using intracellular electrophysiology. Oxidative stress, defined by increased mitochondrial superoxide production and translocation of nitrosylated proteins in enteric neurons, was associated with neuronal apoptosis in colonic segments of oxaliplatin-treated animals. Thus, damage to the enteric neurons induced by oxidative stress might underlie intestinal dysfunction associated with oxaliplatin treatment.

THE IMPACT OF INTERNALISATION ON COMPARTMENTALISED SIGNALLING OF THE DELTA-OPIOID RECEPTOR

Lib En Tiah, Nigel W. Bunnett, Holly R. Yeatman, Daniel P. Poole, Meritxell Canals, Michelle L. Halks

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The delta-opioid receptor (DOPr) is a promising therapeutic target for the treatment of pain and gastrointestinal related disorders. However, the impact of agonist-induced receptor endocytosis on DOPr signalling still remains largely unknown. The importance of receptor trafficking for signal generation was examined in DOPr expressing HEK293 cells using bioluminescence resonance energy transfer (BRET) and Förster resonance energy transfer (FRET). The DOPr agonists SNC80 and DADLE, which strongly and moderately internalise DOPr respectively, stimulated DOPr trafficking from HRas-containing lipid-rich domains of the plasma membrane to Rab5a-positive early endosomes. In contrast, the weakly internalising DOPr agonist, ARM390, only triggered movement of DOPr away from the non-lipid-rich plasma membrane domains. All three agonists induced activation of Gαi1, Gαi2, Gαi3 and Gαi/o but not Gαs or Gαq, suggesting that DOPr only activates Gαi/o G proteins. While all ligands activated G proteins, only SNC80 and DADLE (but not ARM390) induced recruitment of β-arrestins to DOPr. The ligands also induced distinct spatiotemporal signalling profiles. SNC80 induced a transient increase in plasma membrane and cytosolic PKC activity. In contrast, DADLE caused a sustained increase in plasma membrane and cytosolic PKC activity, and there was no effect of ARM390. The distinct temporal profiles were also observed when ERK activity was measured: SNC80 induced transient cytosolic and nuclear ERK, DADLE induced sustained cytosolic and nuclear ERK, while ARM390 only induced sustained cytosolic ERK. These findings suggest a link between DOPr trafficking and compartmentalised signalling. Future studies will determine whether DOPr trafficking controls ligand-dependent differences in spatiotemporal signalling.
**Poster #13**

EFFECTS OF POLYPHARMACY TREATMENT ON INTERSTITIAL CELL OF CAJAL AND NEURONAL NETWORKS IN MICE GASTROINTESTINAL TRACT

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Adverse drug reactions and interactions are particularly prevalent in old age, with adverse effects to the gastrointestinal (GI) tract among the most common. Characterisation of drug related changes in the GI tract are useful for understanding pathological changes in elderly patients with digestive disorders. This study investigates the effects of polypharmacy and hyperpolypharmacy treatment on the neuronal networks and interstitial cell of Cajal (ICC) in adult mice. The role of ICC in motility has been identified as the pacemaker cells for contractile activity of the GI tract. Fixed-frozen sections of the oesophagus and small intestine from three mice populations (control, polypharmacy and hyperpolypharmacy) were immunofluorescently stained with a mixture of antibodies (ICC markers Kit ACK-2, Kit D13A2 and anoctamin 1 (ANO1), and neuronal marker Pgp9.5). The polypharmacy and hyperpolypharmacy treated mice oesophagus showed decreased ICC network volumes in the myenteric plexus, and intramuscular ICCs of longitudinal and circular muscle layers, whereas the small intestine showed no significant ICC changes between populations. The neuronal networks remain unchanged in the myenteric plexi in all samples but were reduced in neuronal processes throughout the circular muscle in the oesophagus and small intestine of both treated populations. Our results indicate a reduction in ICC and neuronal network densities in polypharmacy and hyperpolypharmacy mice. This study suggests a decrease in cellular populations may contribute towards GI dysmotility in elderly patients on multiple drug treatments.

**Poster #14**

ENTERIC NEURAL CELLS FROM HIRSCHSPRUNG DISEASE PATIENTS FORM GANGLIA AUTOLOGOUSLY IN ANEURONAL COLON MUSCLE TISSUE

Dongcheng Zhang¹, Benjamin N. Rollo¹, Lincon A. Stamp², Trevelyan R. Menheniot², Lefteris Statopoulos³, Mark Denham⁴, Mirella Dottori³, Sebastian K. King¹, John M. Hutson¹,²,³ and Donald F. Newgreen¹

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Hirschsprung Disease is caused by failure of neural crest (NC)-derived enteric nervous system (ENS) cells to colonise the distal bowel, resulting in failure of intestinal transit postnatally. Treatment is by distal bowel resection, but neuronal cell replacement may be an alternative. We tested whether aneuronal colon tissue from patients may be colonised by autologous ENS-derived cells. ENS cells were obtained from the proximal resection margin of patient colon using flow cytometry for the NC marker p75. This method was superior to the formation of spontaneous ‘neurospheres’ for isolation of neural lineage cells; which was unable to separate neural from mesodermal cells. Human ENS cells could be cultured but required Wnt agonists for proliferation in vitro, and they could be frozen and thawed successfully. We obtained human aneuronal colon tissue from the distal resection margin of the same patients, and made small (2-4 mm²) explants of smooth muscle tissue for organ culture. Co-cultures of the ENS cells and muscle tissues were established. Cells were assessed for NC markers immunohistochemically and by quantitative (q)RT-PCR, and mitosis was detected by EdU labelling. The donor ENS cells were labelled with Mitotracker-red to enable them to be identified in host tissue. ENS cells derived from Hirschsprung patients by p75 sorting colonised autologous aneuronal colon tissue in co-cultures, proliferating and differentiating as neurons and glia, and extending axons. We conclude that NC-lineage cells can be obtained from Hirschsprung patient colon and can form ENS-like structures in autologous aneuronal colonic muscle.
CHARACTERIZING THE ROLE OF THE CYSTEINE PROTEASE LEGUMAIN DURING CAERULEIN-INDUCED PANCREATITIS USING ACTIVITY-BASED PROBES

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Pancreatitis is an inflammatory disease of the pancreas characterized by dysregulated activity of digestive enzymes, necrosis, immune infiltration, and pain. Repeated incidence of pancreatitis in patients has been identified as an important risk factor for pancreatic cancer. Cysteine cathepsins are proteases that have been implicated in the initiation of pancreatitis through cleavage and activation of trypsinogen. Reducing cathepsin B activity in mouse models of pancreatitis, either through chemical or genetic ablation, results in improved pancreatic histology and reduced systemic effects (e.g. less serum amylase activity).

Legumain, a related cysteine protease, is upregulated during inflammation and has been previously shown to cleave, and possibly activate, cathepsins. We hypothesized that legumain could play a role in initiating pancreatitis upstream of cathepsin activation. We have used fluorescent activity-based probes to monitor legumain activity in caerulein-induced pancreatitis models (both acute and chronic) using whole tissue imaging, fluorescence microscopy, and biochemical analysis. Legumain activity in inflamed pancreas is significantly increased compared to vehicle-treated controls. While legumain activity is highest in cells expressing the macrophage marker CD68, acinar cells also exhibit low levels of active enzyme. We now aim to test whether legumain activity is responsible for initiating the proteolytic cascade and whether legumain-specific inhibitors will have therapeutic efficacy in pancreatitis.

LIDOCAINE-INSSENSITIVE ENTERIC INHIBITORY MOTOR NEURONS ARE INVOLVED WITH MIGRATING MOTOR COMPLEXES IN THE GUINEA-PIG COLON

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The enteric nervous system mediates many intestinal motor patterns including migrating motor complexes in the colon. We used segments of 8-10 cm of small and large intestine taken from guinea-pigs killed humanely. Studies included video recording of gut segments placed in warmed oxygenated Krebs, intracellular electrode recording from the circular muscle and longitudinal muscle strip studies. In empty segments of distal colon, spontaneous migrating motor complexes were blocked by tetrodotoxin (0.6 µM) but not by lidocaine (1 mM). These propagating complexes were also blocked by L-NOARG (100 µM) and by a P2Y receptor antagonist (MRS2179; 10 µM), indicating that enteric inhibitory neurons were involved and still active in the presence of lidocaine. In the small intestine, distension-evoked peristaltic contractions were blocked by both lidocaine and tetrodotoxin, as was cholinergic excitatory transmission to longitudinal muscle strips. Intracellular electrode recording, showed that transmission of the enteric inhibitory neurons, recorded as inhibitory junction potentials, were not affected by 300 µM lidocaine and only minimally reduced by 1 mM lidocaine, but were blocked by tetrodotoxin. Similarly in strips of colon after hyoscine (10 µM), the nerve mediated relaxations evoked by transmural electrical stimulation were not blocked by lidocaine. The results reveal that the action potentials of the enteric inhibitory motor neurons appear to be mediated by lidocaine insensitive, voltage dependent sodium channels. The existence of lidocaine-resistant motor complexes suggest that other enteric neurons, upstream of inhibitory motor neurons, may also utilise a lidocaine-resistant sodium channel.
POSTER SESSION: ENS, cells, molecules

Poster #17

HIGH RESOLUTION NEURONAL IMAGING REVEALS A NOVEL OSCILLATORY FIRING MECHANISM IN THE ENTERIC NERVOUS SYSTEM THAT UNDERLIES MIGRATING COMPLEX GENERATION

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One major mystery of the gastrointestinal tract is how the enteric nervous system coordinates neuronal firing in the different classes of neurons in neighboring ganglia during propagating neurogenic contractions. In this study, we used the latest high resolution, EMCCD camera (Evolve Delta; Photometrics) to visualize temporal activation properties of different morphological classes of myenteric neurons in multiple myenteric ganglia along the mouse colon. Intracellular electrophysiological recordings from the circular muscle confirmed the presence of CMMC's every 2-4 minutes, that propagated along the colon. Each CMMC consisted of repetitive discharge of hexamethonium-sensitive cholinergic excitatory junction potentials (EJPs) producing rapid oscillations in membrane potential at (2Hz) in the circular muscle, lasting ~20s (N=9). To characterize the temporal firing properties of myenteric neurons during these rapid oscillations in the muscle, the calcium indicator, Fluo-4 was loaded into the myenteric plexus. Calcium imaging at 35°C revealed each CMMC was associated with repetitive discharge of synchronized calcium transients in large populations of Dogiel Type-II and Type I neurons across multiple rows of myenteric ganglia and interstitial strands (N=9). The frequency of the repetitive calcium transients in myenteric ganglia (2.0 ± 0.1Hz) and interstitial strands (2.2 ± 0.1Hz) was the same as the cholinergic EJPs recorded at the same time in the smooth muscle. All synchronized calcium transients across multiple ganglia were abolished by tetrodotoxin. This is the first demonstration of a rhythmic neuronal firing pattern in the ENS that is responsible for repetitive cholinergic EJPs in the smooth muscle during a complex intestinal motor pattern.

Poster #18

SEXUAL DIMORPHIC EFFECTS OF CHOLERA TOXIN IN COLONIC MOTILITY ARE MEDIATED VIA ESTROGENS AND SEROTONIN IN FEMALE C57BL/6 MICE

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Substantial evidence indicates that sex hormones influence gastrointestinal motility. More women (2:1) exhibit Functional Bowel Disease (FBD) and often show cycling gastrointestinal complications, which appear or worsen during the postovulatory phase of the ovarian cycle. Furthermore, nutrient transit in the intestine is slower during the lutal phase than in the follicular phase of the ovarian cycle. We have shown that cholera toxin (CT) reduced colonic motility in vitro in randomly selected female C57Bl/6 mice, but did not affect male colon. This inhibition was seen during estrus, but not in proestrus, and was reversed by granisetron (5-HT3 receptor antagonist). CT had no significant effect on TPH1KO mice (selectively lacking mucosal 5-HT). We concluded that the CT effect in female mice depends on estrogens and mucosal 5-HT acting on 5-HT3 receptors. We now report immunohistochemical analysis of estrogen receptors in whole mounts of myenteric plexus and of 5-HT in frozen cross sections of colon. In estrus females 87% of myenteric neurons had significant nuclear expression of estrogen receptor alpha (ERa), while male mice had 61% and proestrus female mice had 41%. Nuclear localization of estrogen receptor beta (ERb) was significantly lower ranging from 19-26% with no significant differences between groups. When 5-HT positive GC cells were counted in cross sections from mid colon, estrus females had more (29±13) than proestrus females (22±11) or males (19±17). Thus both estrogens and 5-HT are likely to be involved in CT mediated motility effects.
Poster #19

ASSESSMENT OF GASTRIC ACCOMODATION BY INTRAGASTRIC PRESSURE MEASUREMENT DURING DIFFERENT NUTRIENT DRINK INFUSIONS

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Intragastric pressure (IGP) measurements, used to assess gastric accommodation, show that intragastric nutrient infusion induces an initial drop in IGP, followed by a gradual recovery with significant correlation to satiation. The objectives were to assess the role of different macronutrients in inducing this IGP response. A manometry and infusion catheter were positioned in the proximal stomach of 15 healthy volunteers (21-52y, 7men). Three nutrient drinks (lipid [10% triglyceride emulsion], protein [whey protein isolate], carbohydrate [maltodextrin]) were intragastrically infused at 60mL/min, in a single-blind randomised order until maximum satiation (100mmVAS). On separate occasions, gastric emptying (GE) of each drink was determined using the [14C]octanoate breath test, where exhaled breath was analysed for 13CO2 and 50% GE (t/5min) calculated. Results showed volunteers scored maximal satiation after a higher volume of the carbohydrate (121±126mL) compared to the other nutrients (protein 1049±99mL, lipid 1006±49mL; p=0.0027). In all subjects after each nutrient, the IGP decreased initially to gradually recover thereafter. Maximum IGP decrease (termed nadir) was 7.6±0.9mmHg after 8.1±1.3min, 9.4±1.0mmHg after 10.1±1.2min, and 7.7±0.6mmHg after 8.7±1.2min for carbohydrate, protein and lipid respectively (nadir p=0.1461; time to nadir p=0.4422). Post hoc analysis showed significant correlations between IGP and satiation score increase. The carbohydrate drink emptied significantly faster (40±13 t/5min) compared to the protein and lipid (82±10 and 106±16 t/5min, respectively). These findings indicate all three macronutrients induce an IGP response in the healthy state, giving an opportunity to identify whether these nutrients drive the impaired gastric accommodation seen in functional dyspepsia.

Poster #20

RELATIONSHIP BETWEEN FATTY ACID TRANSPORTER EXPRESSION AND SUPPRESSION OF ENERGY INTAKE FOLLOWING INTRADUODENAL LIPID INFUSION IN HEALTHY HUMANS

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Small intestinal fat stimulates pyloric motility and suppresses energy intake (EI), mediated by the release of hormones from enteroendocrine cells (EEC), which occurs via stimulation of fatty-acid receptors (FAR) and FA transporters (CD36) on EEC. Potential relationships between changes in FAR/CD36 expression following acute fat exposure with motility and EI responses are unknown. We investigated the effects of acute intraduodenal (ID) lipid infusion on FAR/CD36 expression and isolated pyloric pressure waves (IPPWs). 20 fasted healthy, lean volunteers (BMI: 21.6±1.4 kg/m²) were studied on 2 days: (1) to evaluate the effects of ID lipid (10% Intralipid®; 2kcal/min for 120 min) on IPPWs (manometry) and EI and fat intake at an ad libitum buffet-meal, and (2) to collect duodenal biopsies at baseline and post-ID lipid infusion (endoscopy). Relative expression of FARs (FFAR4, FFAR1, GPR119) and CD36 was determined by RT-PCR, with normalised data presented. ID fat infusion revealed two distinct groups: “responsive” participants (n=13) with measurable IPPWs, and “non-responsive”, where no IPPWs occurred (n=7). In the overall group, IPPWs, EI and total fat consumption did not correlate with changes in FAR, or CD36 expression, and expression of FARs and CD36 did not differ between groups. However, decreased CD36 expression was significantly correlated with suppression of EI and reduced total fat consumption (both r=-0.592, P<0.05), in “responsive”, but not in “non-responsive”, participants. We propose that altered intestinal expression of CD36 induces signalling to enhance pyloric motor and EI responses to fat. Further work is needed to determine the mediating effects of gut hormones.
THE EFFECTS OF PROTEIN ON BLOOD GLUCOSE CONCENTRATIONS AND GASTRIC EMPTYING IN HEALTHY YOUNG AND OLDER SUBJECTS

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Protein-rich supplements are used widely for prevention and management of malnutrition in elderly. Ingestion of a bolus of proteins, rich in insulinotropic amino acids, may result in a drop in blood glucose concentrations, particularly in older people. The randomized double-blind study aimed to determine the effect of ageing on glucose-concentration responses and gastric emptying of protein loads. In 8 young (25±6yrs, 72±8kg, 23±2kg/m²) and 8 healthy older (73±4yrs, 77±11kg, 26±4kg/m²) men, glucose-concentration responses (glucometer) and gastric emptying (3D-ultrasonography) of a control drink (~40kcal) and 120 and 280kcal whey-protein drinks (450ml) were measured (0-180min). Baseline glucose was not different between study-days and age-groups (young: 5.37±0.19mM; older: 5.39±0.15mM; P=0.05). In older subjects, the nadir of glucose concentrations, which was reached at 90min after the 280kcal-protein drink, was lower compared to control (280kcal-protein vs. control: -1.0±0.2mM vs. -0.4±0.1mM; P<0.05); whereas in young subjects there was no significant difference in the nadir of glucose after both protein loads compared to control (P=0.05). AUC glucose concentrations were lower after both protein loads compared to control in both age-groups (control, 120, 280kcal; young: -14±33, -76±15, -60±16mmol/L; older: 28±33, -25±14, -71±20mmol/L; protein-load effect P=0.026). The age-effect on glucose was not significant (P=0.147). After ingestion of all 3 drinks, gastric emptying was slower in older than young (mean T50 of 3 study days; young vs. older: 36±5min vs. 68±5min; main age-effect P<0.007). The slower gastric emptying of protein in older compared with young adults may be favourable to prevent a potential drop in glucose after protein ingestion.

ATTENUTED POSTPRANDIAL BLOOD GLUCOSE RESPONSE AND DELAYED GASTRIC EMPTYING ARE IMPROVED WITH REFEEDING IN ANOREXIA NERVOSA

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The rate of gastric emptying (GE) is an important determinant of postprandial blood glucose (BG) concentrations, yet GE is delayed in anorexia nervosa (AN). This study aimed to characterise GE and BG responses to a mixed-nutrient meal in untreated AN on admission and following 1 (W1) and 2 weeks (W2) of refeeding, and to examine the relationship between GE and BG responses. In 22 female adolescent AN in-patients and 17 age-matched healthy controls (HC), GE (¹³C-octanoate breath test) of, and BG response to, an oral semi-solid test meal was evaluated over 120 min. Compared with HC, GE was markedly delayed in AN on admission (AN: 192±21, HC: 310±40%/hr, P<0.01), while faster (AN: 297±34), and no longer different from HC, at W2. While fasting BG did not differ between AN on admission and HC, the postprandial rise was absent in AN (AN: 635±14, HC: 803±29 mmol/L.min⁻¹, P<0.01), and, at 60 min, BG decreased below baseline in AN (P<0.01). At W2, postprandial BG increased in AN, yet remained lower than in HC (AN: 713±18, P<0.05). Furthermore, there was a moderate correlation between GE and BG in HC (R²=0.643, P<0.01), but not in AN. In conclusion, our data indicate that (1) GE of, and BG response to, a mixed-nutrient meal are markedly impaired in untreated AN, and (2) nutritional rehabilitation may restore, at least in part, the gut responses to nutrients. Further research is required to elucidate the mechanisms underlying altered GE and postprandial BG responses in AN.
COMPARATIVE EFFECTS OF INTRADUODENAL PROTEIN ON GUT MOTILITY, HORMONE RELEASE, GLYCEMIA, APPETITE AND ENERGY INTAKE IN LEAN AND OBESE MEN

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In lean individuals, intraduodenal protein and lipid load-dependently modulate gastrointestinal motor and hormone functions and reduce energy intake (EI); protein also stimulates insulin, with modest effects on blood glucose. Gastrointestinal sensitivity to intraduodenal lipid is diminished in obesity; whether gastrointestinal sensitivity to protein is also reduced remains unclear. We assessed the effects of increasing loads of intraduodenal protein on antropyloroduodenal motility, gastrointestinal hormone release, glycaemia and EI in obese individuals, and compared the effects of an equicaloric load in lean and obese subjects. For this purpose, we measured in 12 non-diabetic obese males, on 3 occasions, in double-blind, randomised order, antropyloroduodenal pressures, plasma glucagon-like peptide-1 (GLP-1), cholecystokinin, glucagon-dependent insulinotropic polypeptide (GIP), glucagon, insulin and blood glucose, during 60-min intraduodenal infusions of protein at either i) 1.5 or ii) 3 kcal/min, or iii) saline control. 12 age-matched lean individuals received a 3 kcal/min infusion only. Immediately after the infusions, EI from a buffet-lunch was quantified. In obese subjects, protein load dependently suppressed antral and duodenal pressures, stimulated cholecystokinin, GLP-1, GIP, insulin and glucagon (all r=0.57, P<0.01), and reduced EI (r=-0.38, P=0.057). EI was marginally higher in the obese (P=0.08). Motility, cholecystokinin, GLP-1 and glucagon responses did not differ between lean and obese. The effect of protein on blood glucose was also comparable, despite a larger insulin, and smaller GIP, release in the obese (both P<0.05). Thus, GI sensitivity to protein remains relatively intact in obesity, however, the observed changes in insulin and GIP suggest that mechanisms involved in glycemic control may be disturbed.

LOAD-DEPENDENT EFFECTS OF ORAL PROTEIN ON GASTRIC EMPTYING, GLYCAEMIA AND ENERGY INTAKE IN HEALTHY MEN

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In healthy, lean individuals, intraduodenal whey protein stimulates pyloric motility and insulin release, while maintaining normoglycaemia, and suppresses energy intake (EI), in a load-dependent manner. Whether the load-dependent effects of protein are maintained when protein is consumed orally, thus, involving ‘gastric’ mechanisms, is unclear. We hypothesized that oral protein would load-dependently slow GE, modulate postprandial glycaemia and suppress EI. 18 lean males (age 24.7±3 yr, BMI 22±1 kg/m²) received, on 3 separate occasions, in randomized, double-blind order, isosmolar, equally-palatable drinks (~450 ml), containing either 30 (L) or 70 (H) g whey protein, or saline (control). Immediately after the drink (t=0 min), GE (assessed using 3D ultrasound), plasma insulin and blood glucose were measured at 15-min intervals for 180 min. EI was measured at a buffet-style lunch (t=180-210 min). Both L and H slowed GE, and GE was slower following H compared with L (T½(min); control: 12±1, L: 26±3, H: 65±9; all P<0.001). Protein load dependently stimulated insulin release (P<0.01), but glucose concentrations were modestly reduced only with H between t=45-150 min compared with baseline (P<0.05). EI was suppressed by both L and H compared with control (P<0.05) (kcal; control: 1174±91, L: 1027±81, H: 997±71), with no significant difference between L and H. In conclusion, the observation that L and H had comparable effects on energy intake suppression suggests that a threshold of ~30 g of oral whey protein is adequate to suppress subsequent energy intake, while maintaining normoglycaemia in healthy men.
EFFECTS OF INTRADUODENAL L-LEUCINE ON ANTRALDUODENAL MOTILITY, GUT HORMONE, INSULIN AND GLUCAGON RELEASE, BLOOD GLUCOSE AND ENERGY INTAKE IN HEALTHY MEN

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There is increasing evidence that the branched-chain amino acid, L-leucine, has potent glucoregulatory and eating-inhibitory effects. We hypothesized that these effects are mediated via changes in gut motor and hormone functions, and investigated the effects of L-leucine on antropyloroduodenal motility, plasma gut hormone (CCK, PYY, GLP-1), insulin, glucagon and L-leucine, and blood glucose, concentrations, and energy intake. 12 healthy, normal-weight men were studied on 3 occasions in randomized, double-blind fashion. Antropyloroduodenal motility and blood/plasma parameters were measured during 90-min intraduodenal infusions of L-leucine at 0.15 (~14 kcal) or 0.45 (~41 kcal) kcal/min, or saline (control). Energy intake was quantified at a buffet meal following the infusion. L-leucine at 0.45 kcal/min, but not at 0.15 kcal/min, suppressed antral pressures (P<0.05), but did not stimulate pyloric pressures. L-leucine at both 0.15 and 0.45 kcal/min moderately stimulated plasma CCK and insulin (both P<0.05), but did not affect plasma PYY, GLP-1 or glucagon, concentrations. L-leucine substantially increased plasma L-leucine in a dose-related manner (P<0.001). L-leucine at 0.45 kcal/min, but not at 0.15 kcal/min, also reduced blood glucose, which, however, remained within the normoglycaemic range, and substantially reduced energy intake, in excess of the caloric content of the infusion (by 156±48 kcal vs. control, P<0.05). In conclusion, our data suggest that the effects of low intraduodenal loads of L-leucine on energy intake and blood glucose are not primarily mediated via activation of GI functions and glucoregulatory hormones, but that direct effects of circulating L-leucine, possibly within the brain, may play an important role.

REGIONAL VARIATION IN EXPRESSION OF ENZYMES THAT SYNTHESISE INCRETIN HORMONES AND GLUCAGON IN LEAN AND MORbidLY OBese HUMANS

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We recently showed that enteral glucose-induced release of glucagon and glucose-dependent insulinotropic polypeptide (GIP) is increased in morbidly obese humans, while glucagon-like peptide-1 (GLP-1) release is decreased - changes that promote hyperinsulinaemia and hyperglycaemia. Jejunal expression of prohormone convertase-1 (PCSK1, the proteolytic enzyme for GIP and GLP-1 production) is also decreased in morbidly obese patients with type 2 diabetes compared to those without diabetes which may contribute to hyperglycaemia. However, regional expression of PCSK1, and of PCSK2 (the proteolytic enzyme for glucagon production) has not been evaluated in relation to obesity. Endoscopic biopsies were collected from the duodenum of lean and obese subjects while left colon biopsies were collected from separate lean and obese subjects, none of whom had diabetes. PCSK1 and PCSK2 transcript levels were assessed by qPCR. PCSK1 transcripts were 5-fold higher (P<0.001) in duodenum than left colon of lean subjects, while PCSK2 transcripts - present at half the level of PCSK1 in the duodenum (P<0.001) - were 8-fold higher in duodenum than left colon (P<0.001). There were no differences in PCSK1 or PCSK2 transcript distribution or abundance between lean and morbidly obese subjects. In conclusion: (i) duodenal L-cells may have greater capacity for incretin hormone biosynthesis and release, despite greater L-cell density in colon; (ii) the relatively high level of duodenal PCSK2 expression supports the concept of gut-derived glucagon production in humans; and (iii) transcriptional differences in PCSK1 or 2 do not underlie reduced GLP-1 production or augmented glucagon release in human obesity.
DO COLONIC MIGRATING MOTOR COMPLEXES (CMMCs) OCCUR IN MICE LACKING THE EDN3 GENE?

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In mammals, colonic migrating motor complexes (CMMCs) are a major propulsive contraction responsible for the expulsion of fecal content. Mice with a mutation of the endothelin-3 (EDN3) gene raised on a 129SL background strain have ~70% colonic aganglionosis, lack CMMCs and are lethal within 12 days postpartum. In contrast, EDN3 mutant mice raised and maintained on a C57BL6 background strain (lethal-spotted, Is/Is mice) can live for much longer periods but it is unclear whether CMMC generation is preserved in these mice also lacking the EDN3 gene. The aim of this study was to determine whether CMMCs exist in Is/Is mouse colon; and if so, whether their existence and frequency is related to the length of aganglionosis. Spatio-temporal mapping and mechanical recordings of colonic wall movements were made from isolated whole colons obtained from wild type and Is/Is mice. Although Is/Is mice had megacolon, they still generated CMMCs in the ganglionic segment; which on some occasions could propagate short distances into the aganglionic region. There was large variability in the length of aganglionosis, which showed weak correlation with the existence or frequency of CMMCs. Interestingly, CMMC propagation velocity was slower in Is/Is mice when evoked by intraluminal fluid. A myogenic motor pattern was identified in the aganglionic region that was maintained under tonic inhibition. We show that despite megacolon, Is/Is mice still generate CMMCs in the ganglionic region. These offspring have sufficient propulsive motility in the ganglionic segment to live a normal murine lifespan and rarely die of bowel obstruction.

SPATIO-TEMPORAL ANALYSIS OF NEUROGENIC STATES DURING PROPULSIVE MOTOR ACTIVITY IN GUINEA-PIG SMALL INTESTINE, DISTAL COLON AND RAT COLON

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Intestinal distension of isolated segments of small and large intestine of experimental animals elicits neurally dependent peristaltic contractions. We used a novel method to establish indirectly the activity of excitatory and inhibitory motor neurons during motor behaviour (Costa et al 2013 Frontiers in Systems Neuroscience. Hypothesis and Theory Article, 7, 1-18.) in the guinea-pig small intestine and distal colon and in the rat colon (n=4 each). The animals were killed humanely (permit Flinders University AWC). Segments of 10-12 cm of intestine were placed in warmed oxygenated Krebs, cannulated orally for intraluminal infusion of Krebs and expulsion of contents via an aboral cannula. Video and intraluminal pressure recordings were used to construct spatio-temporal maps of active and passive mechanical states of the muscle. In the guinea-pig small intestine peristalsis is due to an uninterrupted area of activity of excitatory motor neurons traveling aborally. In the guinea-pig distal colon infusion of liquid elicited either pellets like boluses of multiple peristaltic contractions, each being due to similar areas of activation of excitatory motor neurons traveling aborally along the entire segment. In the entire rat colon distension resulted in multiple incomplete peristaltic contractions. Plotting the active contractions revealed that there is only one area of activity of excitatory motor neurons traveling all the way from the oral end of the proximal colon to the anal end of the distal colon. Our approach opens a new depth of analysis in establishing the relative roles of myogenic and neurogenic mechanisms during complex intestinal motor events.
TRACING GASTROINTESTINAL TRANSIT IN PHARMACOLOGICAL MODELS OF CHRONIC DYSMOTILITY IN AGED RATS

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We developed two pharmacological rat models of chronic dysmotility for research on conditions of constipation or hypermotility relevant to the aged. The serotonin agonist prucalopride was used to induce hypermotility, and the opioid agonist loperamide to induce constipation. We tested whether chronic administration of these modulatory drugs over seven days would alter gastrointestinal tract (GIT) transit. To determine how GIT transit of solids might be affected in different regions, transit of metallic beads was tracked over 12 hours. Male rats (18 months) were given 1, 2, or 4 mg/kg/day prucalopride or loperamide (in DMSO) for 7 days by subcutaneous slow release capsule (10 μl/hour), and controls DMSO vehicle only. Six solid metal beads (d=1.4 mm) were gavaged with 15% barium sulphate. GIT transit was tracked at 4, 9, and 12 hour time points by high resolution X-ray imaging. Procedures were carried out under brief isoflurane anaesthesia. A rating scale was used to classify GIT bead location in vivo (Reed et al., Neurogastroenterology & Motility 2014, 26:1663-68) and distance of beads from the caecum measured post-mortem. Loperamide (1 mg/kg) slowed GIT transit at 9 and 12 hour time points. Prucalopride did not significantly alter GIT transit scores, but at 4 mg/kg beads moved significantly further from the caecum in 12 hours than in controls. Our results indicate that loperamide slowed GIT whereas prucalopride increased the rate of colonic transit, providing models useful for studying the effects of diet on constipation and hypermotility.

INTRATHecal APPLICATION OF NORAdeNAline CAUSes PROPULsive CONTRACTIONS OF THE COLORECTUM IN ANAESTHETIZED RATS

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Defecation is controlled by neural circuits that involve both the enteric nervous system and the central nervous system (CNS). The enteric nervous system directly controls smooth muscles and is regulated by the CNS to control fecal continence. In the CNS are two defecation centers, the lumbosacral spinal defecation center and a center in the brain stem; these two centers communicate with each other. However, mechanisms through which the centers communicate are poorly understood. In the brain stem are abundant monoaminergic neurons, some of which project to the region of the defecation center in the lumbosacral spinal cord. In this study we focused on noradrenaline (NA), the major neurotransmitter of descending monoaminergic neurons. Rats were anaesthetized with alpha-chloralose and ketamine, and both the distal colon and anus were cannulated to measure intra-colorectal pressure and expelled intraluminal liquid volume. Intrathecal application of NA (10 μL of 5M) at the level of the lumbosacral defecation center caused strong propulsive contractions of the colorectum. Pre-application of tetrodotoxin to the lumbosacral spinal cord blocked the effect of intrathecally applied NA. The alpha-1 catecholamine receptor agonist phenylephrine applied intrathecally mimicked the effect of NA and the alpha-1 receptor antagonist prazosin, applied intrathecally prior to NA, blocked its effect. The effect of NA no longer occurred after bilateral severing of the pelvic nerves. Our results demonstrated that intrathecal NA activates the sacral parasympathetic preganglionic neurons. The results indicate that NA could mediate signaling from the brain stem to the lumbosacral spinal defecation center.
INVESTIGATION OF THE PHYSIOLOGICAL ROLES OF GHRELIN RECEPTORS IN CONTROL OF DEFACTION

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In species so far investigated (dog, guinea-pig, human, mouse, rat), agonists of ghrelin receptors, that cross the blood / CNS barrier, increase colorectal propulsion and cause defecation. This effect is exerted within the spinal cord defecation centre. However, ghrelin itself, the only known natural ligand for the conventional ghrelin receptor (GHSR1a) is not found in the spinal cord. Moreover, other, currently molecularly undefined, receptors for ghrelin family ligands exist. This raises two questions, first whether effects of agonists are reduced by antagonists of GHSR1a and second whether defecation that is behaviourally evoked is reduced by a GHSR1a antagonist. To investigate receptor pharmacology, we used anaesthetised rats to deliver compounds directly to the defecation centre (i.t.) or intravenously (i.v.). I.t. delivery of ghrelin (1 μM) or the GHSR1a agonist, ulimorelin (5 μg) caused colorectal propulsion. The centrally penetrating antagonist, YIL782 (3 mg/kg bolus plus 5 mg/kg/h) blocked the effect of i.t. ulimorelin. The effect of i.t. ghrelin was blocked by i.t. tetrodotoxin (0.05 μg). We used water avoidance (WA) in conscious rats to activate the descending pathways impinging on the defecation centre. Rats were placed on a small platform surrounded by water and pellets expelled were counted each 10 min for an hour. There was a substantial increase in pellets in 10 minutes after WA, which was reduced to < 20% by YIL781 (3 mg/kg i.p. 10 mins before WA) compared to vehicle control. Results imply that there is an unidentified ghrelin-like endogenous transmitter in the lumbosacral cord that stimulates defecation.

RESTORATION OF GASTROINTESTINAL FUNCTION IN MPTP MODEL OF PARKINSON’S DISEASE

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Patients with Parkinson’s Disease often experience non-motor symptoms including constipation, which have a significant impact on quality of life. Understanding the cause of these early non-motor deficits is essential for developing disease modifying therapeutic strategies that could prevent disease progression. Specific neuronal subpopulations were reduced within the myenteric plexus of mice 21 days after lesioning through the intraperitoneal administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and was concomitant with a reduction in stool frequency, indicative of gastrointestinal dysfunction. Oral administration of Cu²⁺ (atm) which, has been shown to be neuroprotective and restore motor performance to MPTP lesioned mice, improved stool frequency and was correlated with restoration of neuronal subpopulations in the myenteric plexus of MPTP lesioned mice. Restoration of gastrointestinal function was associated with reduced enteric glial cell reactivity and reduction of markers of inflammation. Therapeutics that have been shown to be neuroprotective in the central nervous such as Cu²⁺ (atm) therefore also provide symptom relief and are disease modifying in the gastrointestinal tract and indicate a common cause of pathogenesis in the enteric and central nervous system.
NON-INVASIVE MEASUREMENT OF GASTRIC EMPTYING AND ORAL-RECTAL TRANSIT IN YOUNG PIGLETS

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It would be useful to have a large animal model to study changes in gastrointestinal motility. Aim: To develop a non-invasive method of estimating gastric emptying and oral-rectal transit times in young piglets. Methods: We performed liquid and solid marker transit studies in 4 week-old, Large White female pigs. Ten animals (5.7 ± 0.3kg (mean ± SEM)) were fed blue-dyed grower feed and monitored by video surveillance. Twenty-two animals (7.7 ± 0.59kg) were administered 18-mm-diameter radio-opaque plastic markers (sitz markers) under light anaesthesia (5% isoflurane) and had abdominal x-rays taken at 6,30,54,78 hours. Transit was calculated using the compartmental method used in humans. Results: Using blue dye (fluid transit), the median (25th, 75th percentiles) time to first incidence of blue-dyed stool was 13.2 (10.2, 18.1) hours and to last blue stool was 24.1 (22.4, 40.3) hours. Plastic markers were evacuated between 30 and 80+ hours. Median oral-rectal transit time was 25.2 (17.8, 40.5) hours. The marker method was able to separate gastric emptying and showed that pigs were able to hold solids in the stomach for extended times with gastric transit times from 1-80 hours. Conclusion: Fluid-phase markers appeared earlier than solid markers. Using plastic markers and x-rays to estimate the segmental and oral-rectal transit times in young pigs may be a useful method that can be correlated to oral-rectal transit studies performed in humans. The ability of pigs to hold solids in the stomach for extended times complicates transit studies.

EFFECTS OF TRPA1 AGONISTS ON SHORT CIRCUIT CURRENT IN THE MOUSE DUODENUM AND COLON

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TRPA1 receptors are ligand-binding cation channels, commonly associated withafferent nerve endings that have a wide distribution. Several phytochemicals in food activate TRPA1. These include allyl isothiocyanate (AITC) from mustard and wasabi, cinnamaldehyde from cinnamon, and linalool, for example from mint. We previously reported that 5-HT containing enteroendocrine cells express Trpa1 in the mouse duodenum, but not colon. If these are functional receptors, TRPA1 agonists should trigger the release of 5-HT, which would then stimulate chloride ion secretion resulting in an increase in short circuit current (Isc). We have investigated effects of TRPA1 agonists on the Isc of mouse duodenum and distal colon mounted in Ussing chambers, and calcium mobilisation in HEK cells transfected with rTRPA1. The agonists increased Isc in the duodenum with a rank order potency AITC > cinnamaldehyde > linalool (0.1 to 300 μM; n ≥ 6). The same rank order was observed in the transfected cells (n ≥ 4). The rank order was similar in the colon, except that linalool was ineffective at the highest dose (300 μM). Responses to AITC were reduced by the TRPA1 antagonist, HC-030031 (100 μM; n ≥ 6), and were greatly diminished in TRPA1−/− tissues (n ≥ 6) in both the duodenum and colon. The 5-HT3 receptor agonist, caragesten (1 μM) did not reduce responses. It is concluded that there are functional TRPA1 receptors in the duodenum, where in situ hybridisation has revealed Trpa1 expression in enteroendocrine cells, and also in the colon where such expression has not been observed.
QUANTITATIVE IMMUNOHISTOCHEMICAL CO-LOCALIZATION OF TRPV1 AND CGRP IN VARICOSE AXONS OF THE MURINE OESOPHAGUS, STOMACH AND COLORECTUM

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In the gastrointestinal (GI) tract of mammals, spinal afferent neurons, with cell bodies in dorsal root ganglia (DRG) detect sensory stimuli, including those that give rise to pain. Many of these neurons express calcitonin gene-related peptide (CGRP) and transient receptor potential cation channel subfamily V member 1 (TRPV1) in their cell bodies and axons, which has led to the use of CGRP and TRPV1 as peripheral markers of spinal afferent neurons. Although CGRP and TRPV1 are known to coexist in some axons of spinal afferent neurons in the GI tract, their degree of coexistence along its length has yet to be quantified. In this study, we used double-labeling immunohistochemistry to quantify the coexistence of CGRP and TRPV1 in varicose axons of the murine oesophagus, stomach and colorectum. We observed that the majority of CGRP-immunoreactive (IR) varicosities within myenteric ganglia of the lower oesophagus (97 ± 1%), stomach (95 ± 1%) and colorectum (91 ± 1%) were also TRPV1-IR. Similarly, the majority of TRPV1-IR varicosities within myenteric ganglia of the lower oesophagus (95 ± 1%), stomach (91 ± 1%) and colorectum (96 ± 1%) were also CGRP-IR. Antiserum to TRPV1 or CGRP did not label myenteric nerve cell bodies at any of the sites studied. Our observations reveal that in the murine oesophagus, stomach and colorectum, CGRP and TRPV1 are almost exclusively expressed together in the axons of spinal afferent neurons.
IDENTIFICATION OF DIFFERENT TYPES OF SPINAL AFFERENT NERVE ENDINGS THAT ENCODE NOXIOUS AND INNOCUOUS STIMULI IN THE STOMACH AND ESOPHAGUS USING A NOVEL ANTEROGRADE TRACING TECHNIQUE

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The nerve endings of spinal afferents innervating the oesophagus and stomach have not been identified. We have recently developed an anterograde tracing technique which facilitates selective labeling of only spinal afferent axons and their nerve endings in visceral organs. Mice were anesthetized, thoracic DRGs (T8-T12) surgically exposed, then injected with dextran-amine. Seven days postsurgery, the entire stomach and oesophagus was removed, fresh fixed and stained for CGRP. The characteristics of 7 major types of spinal afferent nerve endings were identified throughout the corpus, antrum and fundus of stomach of 11 control C57BL/6 mice. The greatest proportion of nerve endings was in the myenteric ganglia (44%), and circular muscle (23%). These nerve endings consisted of fine intraganglionic varicose endings (IGVEs) that ramified through multiple myenteric ganglia, very similar to the IGVEs we recently identified in the colorectum. Greater than 90% of IGVEs and “simple” type varicose nerve endings in the CM layer were CGRP-positive (N=10). No intraganglionic laminar endings (IGLEs) were observed that resembled those reported for vagal afferent endings. The other types of spinal afferent endings consisted of “simple” fine varicose nerve endings that innervated intermodal strands, the submucosa and mucosa, blood vessels and longitudinal muscle (N=10). Spinal afferent endings were rare in the esophagus, but in 2 animals consisted on “simple” type fine varicose endings that ramified within the skeletal muscle and/or myenteric ganglia. We present the first complete characterization of the different types of spinal afferent nerve endings that innervate the upper gastrointestinal (GI) tract of a mammal. The findings reveal a complex array of different types of primary afferent endings that innervate specific layers of the stomach. Some of the novel classes of nerve endings identified must underlie the transduction of noxious and/or innocuous stimuli from the upper GI-tract in vivo.

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CHRONIC ACTIVATION OF THE GC-C/CGMP PATHWAY BY LINACLOTIDE INHIBITS ASCENDING NOCICEPTIVE PATHWAYS AND RESTORES ABERRANT SPINAL CORD SIGNALING

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Linaclotide, a guanylate cyclase-C (GC-C) agonist, reduces abdominal pain and improves constipation in patients with irritable bowel syndrome with constipation (IBS-C). We have recently shown that acute application of linaclotide inhibits colonic nociceptor mechanosensitivity with greater efficacy during chronic visceral hypersensitivity (CVH). However, the effects of chronic linaclotide administration on nociceptor function remain to be determined. We investigated healthy C57BL/6 mice and mice with CVH, 28 days post-TNBS administration. Mice from each group were randomly assigned to either chronic linaclotide (3µg/kg/day) or placebo (water) administration, consisting of a once daily oral gavage for 2 weeks prior to experimentation. In healthy mice chronic treatment with linaclotide significantly reduced nociceptor mechanosensitivity compared with healthy mice treated with placebo. Colonic nociceptors from CVH mice displayed mechanical hypersensitivity and reduced mechanical activation thresholds. Chronic treatment with linaclotide significantly reduced CVH colonic nociceptor mechanosensitivity compared with CVH mice treated with placebo. Furthermore, chronic treatment with linaclotide reversed the reduced mechanical activation thresholds of nociceptors from CVH mice compared to placebo. In separate experiments, chronic linaclotide administration to CVH mice significantly reduced the signaling of in vivo noxious colorectal distention (CRD) to the TL spinal cord compared with CVH mice administered placebo. Chronic oral administration of linaclotide reduces colonic nociceptor mechanosensitivity and reduces nociceptive signaling within the spinal cord in response to noxious CRD, with greatest effect during CVH. These results complement our previous findings in mice and provide further mechanistic insight into how linaclotide, through GC-C agonism and the release of cGMP from mucosal epithelial cells, reduces nociceptive signaling from the colon.
TRPV3 CONTRIBUTES TO CHRONIC VISCERAL MECHANICAL HYPERSENSITIVITY

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Transient receptor potential (TRP) channels are key regulators of visceral mechanosensitivity. TRPV3 is implicated in cutaneous sensitization and hyperalgesia, however, its role in visceral sensation and hyperalgesia remain unclear. We determined if TRPV3 contributes to normal colonic mechanosensation and/or to the mechanical hypersensitivity observed in an animal model of chronic visceral hypersensitivity (CVH). A TRP channel expression panel revealed TRPV3 as the most abundantly expressed TRP channel in the colonic mucosa. Furthermore, we also found TRPV3 expression in thoraco-lumbar DRG neurons, where its expression was increased in CVH mice. Pharmacological activation or inhibition of TRPV3 did not affect the mechanosensitivity of healthy nociceptors. However, in CVH mice, TRPV3 activation with farnesylpyrophosphate significantly increased the mechanosensitivity of the already hypersensitive CVH nociceptors. Finally, TRPV3 inhibition with 17(R)-Resolvin D1 significantly reversed the baseline mechanosensitivity displayed by CVH nociceptors. Our data show that TRPV3 is highly expressed in the colonic mucosa and in TL DRG neurons. Notably, TRPV3 expression is up-regulated in DRG neurons from CVH mice. Correspondingly, pharmacological intervention of TRPV3 alters nociceptor mechanosensitivity, but only during CVH. As inhibition of TRPV3 reverses the mechanical hypersensitivity observed in CVH, this suggests TRPV3 plays a specific and key role during CVH. This contribution could be mediated directly, by expression of TRPV3 on colonic nociceptors, indirectly via mucosal TRPV3 regulated mediator release, or via a combination of both mechanisms. As such, targeting of TRPV3 may be beneficial in the treatment of chronic disorders affecting the gut.

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EXTRACELLULAR CGMP, THE DOWNSTREAM MEDIATOR RELEASED IN RESPONSE TO LINACLOTIDE-INDUCED ACTIVATION OF GUANYLATE CYCLASE-C (GC-C), REDUCES EXCITABILITY OF MURINE AND HUMAN DORSAL ROOT GANGLION NEURONS

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Linaclotide, a guanylate cyclase-C (GC-C) agonist, reduces abdominal pain and improves constipation in patients with Irritable Bowel Syndrome with constipation (IBS-C). Cyclic GMP (cGMP) is a second messenger produced in intestinal epithelial cells in response to GC-C activation. We have recently shown that both linaclotide and exogenous extracellular cGMP inhibit colonic nociceptor mechanosensitivity with greater efficacy during chronic visceral hypersensitivity (CVH). However, the effects of exogenous cGMP on sensory neuron function remain to be determined in isolation. Colonic DRG neurons from CVH mice displayed a significantly reduced rheobase and fired significantly more action potentials compared with healthy mice. In a subpopulation of colonic DRG neurons, cGMP inhibited the neuronal excitability of putative nociceptors, significantly increasing the rheobase and reducing action potential discharge. This effect was evident in both healthy and CVH DRG neurons, was most apparent in CVH DRG neurons and occurred at concentrations as low as 100nM cGMP. In human DRG neurons, cGMP induced an overall reduction in the number of cells responding to hypo-osmotic stimulation. In addition, in human DRG neurons cGMP caused, in a dose-dependent manner, up to 60% inhibition of the Ca2+ influx induced by hypo-osmotic stimulation. Exogenous cGMP directly decreases the excitability of sensory DRG neurons isolated from both mice and humans. These results complement our previous findings in mice, which demonstrated that cGMP inhibited the peripheral endings of nociceptors within the wall of the colon. These current findings also provide further mechanistic insight into how linaclotide, through GC-C agonism and the release of cGMP from mucosal epithelial cells, reduces nociceptive signaling from the colon.

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MECHANICAL ACTIVATION OF SPINAL AFFERENTS FROM FLAT SHEETS OF GUINEA PIG DISTAL COLON

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Spinal afferent neurons transduce mechanical stimuli from the gut, giving rise to sensations (discomfort and pain) and reflex responses, via collaterals in the gut, prevertebral ganglia and spinal cord. Several studies have suggested that low-threshold afferents innervating the colon make up a much smaller proportion of afferents than in the rectum. In studies of tubular preparations of guinea pig rectum and colon in vitro we investigated how distension evoked motor patterns activate extrinsic sensory neurons. Contrary to expectations, most sensory traffic from the colon was carried by neurons with low thresholds. To investigate this further, flat sheet preparations were set up in vitro with the mucosa removed and subjected to mechanical stretch while recording afferent activity in colonic nerves. Results (n=10, total 43 single units) indicate that 88% of units responded at one or more sites to von Frey hair probing, and 58% responded to stretch to a wall tension that corresponded to an intraluminal pressure of ~25mmHg. Approximately 65% of units tested (22 of 34) were responsive to capsaicin and 59% of tested units were activated by the nicotinic agonist, DMPP (a feature of enteric viscerofugal neurons). Action potential amplitude and duration varied between classes of afferents, with a significant negative correlation (larger action potentials were faster; p<0.01). Overall, the results indicate that the majority of stretch activated units have thresholds below the equivalent of 25mmHg, even in the colon, where specialised low threshold afferents are reportedly sparse.

DEVELOPMENT OF A NOVEL PREPARATION BASED ON A TRANSGENIC CGRPΔ REPORTER MOUSE TO DIRECTLY CORRELATE FUNCTIONAL PROPERTIES OF COLORECTAL PRIMARY AFFERENT NEURONS WITH THEIR NEUROCHEMICAL PHENOTYPE

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Several neurochemical classes of colorectal afferents have been distinguished, including a large population expressing CGRP. Most electrophysiological recordings from colorectal afferents lack identification of their neurochemical phenotype. We used a novel transgenic mouse, in which functional properties of primary afferents could be directly correlated with their neurochemistry. Thus, we generated a knock-in mouse expressing the fluorescent reporter, mCherry, under the CGRP promoter. Decentralised preparations of L1 and S1 dorsal root ganglia attached to the colorectum were set up in vitro. The bowel was cannulated to apply graded intraluminal distensions, whilst recording intraluminal pressure. Sharp intracellular electrophysiological recordings were made using 5,6-carboxyfluorescein-filled micropipettes to identify nerve cell bodies. Forty-four colorectal afferents were identified by antidromic action potentials evoked by electrical stimulation of the mesentery or low-grade distension (conduction velocity 0.5±0.3m/s, 36 in L1, n=20). Most discharged action potentials to intra-somai depolarization (13/17 tested, rheobase 0.27±0.07mV), showed hyperpolarization-evoked inwardly rectifying I_r-like currents (14/16 cells) and had passive membrane properties typical of DRG neurons (E_m -53±1.4mV, R_m 66±20mΩ). Twenty-two neurons were distension-insensitive (tested to 60cmH2O). Focal electrical stimuli confirmed they had axons in the gut wall (4/4 cells). Seven of 8 distension-insensitive neurons expressed mCherry (max. cross-sectional area 1149±488µm²). Twenty-one of 22 distension-sensitive neurons were low threshold (10cmH2O), firing to 42Hz at 40cmH2O (amplitude: 59.2±8.8mV, half-peak duration: 1.4±0.3ms). Most low-threshold afferents expressed mCherry (14/16 cells, max. cross-sectional area 900±344µm²). In conclusion, we developed a preparation that can rapidly determine functional, electrophysiological and neurochemical phenotypes of colorectal afferent neurons.
MUCOSAL PERMEABILITY CHANGES FOLLOWING DIETS HIGH IN FAT AND ADVANCED GLYCATION END PRODUCTS

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Non alcoholic fatty liver disease (NAFLD) affects up to 30% of the adult population and is now a major cause of liver disease related premature illness and death in Australia. NAFLD is linked to compromised intestinal barrier function and increased intestinal permeability occurs early in NAFLD. Compounds known as advanced glycation end products (AGEs) have been shown to worsen liver injury. AGEs are complexes of reducing sugars and proteins formed when proteins are overheated in the presence of fats and sugars (e.g., by deep frying). From 10 weeks of age, mice were fed four different diets; control, control baked, high fat, and high fat baked for 4, 16, and 33 weeks. Baking the diet at 160°C for 1 hour increases the AGE content five-fold. We investigated changes in body weight, blood glucose, and liver and intestinal histology. Mice were gavaged with FITC-dextran to measure intestinal barrier function in vivo. Epithelial barrier integrity was assessed in Ussing chambers by measuring ionic permeability. Body and liver weight were significantly increased following 16 and 33 weeks in high fat and high fat baked groups compared to controls. Blood glucose was increased following 4 and 16 weeks (high fat and high fat baked) but not at 33 weeks. Following 16 and 33 weeks, ionic permeability and intestinal permeability in vivo were increased in high fat and these were exacerbated in the high fat baked group. These results indicate that diets which are high in fat and AGEs which cause hepatic disease also cause mucosal and permeability changes.
INCREASED CAPACITY FOR INTESTINAL SEROTONIN RELEASE IN OBESE AND DIABETIC HUMANS

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Peripheral serotonin (5-HT) from intestinal enterochromaffin cells (EEC) is an important regulator of gastrointestinal function, hepatic gluconeogenesis, adipose lipolysis and thermogenesis. Increases in plasma 5-HT link to poorer glycaemic control in subjects with type 2 diabetes (T2D), while polymorphisms in tryptophan hydroxylase (Tph1, which synthesises 5-HT) link with human obesity. We assessed glucose-stimulated 5-HT release from the colon and primary ECC in mice, from healthy lean and obese subjects and subjects with type 2 diabetic (T2D), and assessed duodenal Tph1 expression in these, and bariatric (RYGB) subjects. Glucose above 100 mM triggered 5-HT release from mouse colon, with augmented release from individual vesicles shown by single cell amperometry. Tph1 transcript expression was higher in T2D subjects (1.5-fold, P<0.05) and correlated with BMI in non-T2D subjects (P<0.05); transcripts were lower in RYGB compared to obese and T2D (P<0.05). Tph1 transcript levels were unaffected by glycaemic status in lean and T2D subjects, and tended to increase after luminal glucose only in T2D subjects (euglycemia 1.7-fold, P=0.09). Plasma 5-HT levels were higher in morbidly obese subjects with T2D at baseline (P<0.01) and after intraduodenal glucose (AUC, P<0.05); baseline levels correlated positively with BMI across all subjects (P<0.005). In conclusion (i) glucose-stimulated 5-HT release occurs via increased release from single vesicles, (ii) obese and T2D subjects may have increased gut-5-HT capacity, while (iii) gut 5-HT capacity may be lowered following RYGB. These findings support a key role of gut-5-HT in metabolic dysregulation in obesity and T2D.

NUTRIENT SENSING BY ENTEROCROMAFFIN CELLS IN THE GASTROINTESTINAL TRACT

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Enterochromaffin (EC) cells in the gastrointestinal (GI) mucosa provide 90-95% of total body serotonin (5-hydroxytryptamine, 5-HT), and may also act as important nutrient sensors. Luminal glucose and fatty acids augment 5-HT release from EC cells via unknown mechanisms. This affects GI functions such as motility and gastric emptying by stimulating 5-HT receptors on vagal afferent endings lining the GI mucosa. Using pure mouse EC cell cultures, we show that 5-HT release from colonic, but not duodenal, EC cells increases 2.5-fold (±0.47, P<0.05) in response to 300mM glucose. Colonic EC cells also respond to high (300mM) fructose, with a 4-fold (±0.99, P<0.01) increase in 5-HT release. Gene expression analysis of mouse colonic and duodenal EC cells revealed that EC cells differentially express a number of monosaccharide (Sgtl1, Sgl13, Glut1, Glut2, Glut5) and fatty acid (Ffar1, Ffar2, Ffar3, Ffar4, Gpr4, Gpr92, Gpr119) transporter and receptor targets. Interestingly, Ffar3 (5.7±1.4, P=0.016), Glut5 (10.7±2.86, P<0.02) and Glut2 (437.2±235.7, P=0.049) are more highly expressed in EC cells in the duodenum compared to the colon, as is Tph1 (2.4±0.06, P=0.01), the rate limiting enzyme for 5-HT synthesis. In colonic EC cells, Glut1 (2.98±0.6, P=0.01), Ffar2 (8.63±1.35, P=0.01) and Ffar4 (45.3±9.55, P<0.0001) are more highly expressed, further suggesting EC cells may respond differently to nutrients depending on location in the GI tract. By understanding the nutrient sensing mechanisms present in EC cells, we gain important insight into how different nutrients may influence GI functions by augmenting EC cell 5-HT release.
**Poster #47**

**HELCOBACTER PYLORI INFECTION INCREASES 5-HYDROXYTRYPTAMINE RELEASE IN MOUSE STOMACH**

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5-Hydroxytryptamine (5-HT; serotonin) is produced by enterochromaffin (EC) cells which are found in the epithelium of the gastrointestinal (GI) tract. Epithelial 5-HT plays a number of roles, with a recent discovery that it acts as a pro-inflammatory mediator during colitis. However, whether this is true of other regions of the GI tract is unknown. The aim of this study was to measure 5-HT availability in the stomach in a model of Helicobacter pylori gastritis. Female mice (~20g) were killed 14 or 21 days after H. pylori infection and the level of colonisation was determined. Stomachs were pinned mucosa side up in an organ bath and superfused with warm, oxygenated physiological saline. 5-HT release was recorded using carbon fibre amperometry, and local measurements taken from both fundus and antrum. At day 14, <4.5 Log₁₀ colony-forming unit (CFU)/g stomach were retrieved, while at day 21 mice had a >10x higher load (~5.7 CFU/g) and significant inflammatory infiltrates as determined by flow cytometry. Mechanically evoked 5-HT release was detectable in the antrum, and there was a significant increase (P = 0.016, t-test) in 5-HT between infected mice on day 14 (2.34 ± 0.59 µM, n = 4) and day 21 (21.02 ± 7.54 µM, n = 3). In contrast, there was no detectable 5-HT release in the fundus in either condition. These data suggest 5-HT may have a pro-inflammatory role in gastritis, though further studies are needed to determine whether the increased 5-HT is a cause or effect of inflammation.