Future therapies for the treatment of bladder contractile disorders?

Phelps, Charlotte; Chess-Williams, Russ; Moro, Christian

ASCEPT SIG

Virtual National Symposium

Advances in Urogenital & Gastrointestinal Research

Friday 14th October 2022

1-4pm QLD

1.30-4.30pm SA

2-5pm VIC / NSW

PROGRAM & ABSTRACTS

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

AEDT 2.00-2.10	**Welcome – Betty Exintaris / Russ Chess-Williams**	
	SESSION 1: Lightning Snapshots	**Chairs:**
2.10	**Charlotte Phelps, Bond University** *Future therapies for the treatment of bladder contractile disorders?*	Donna Sellers / Iris Lim
2.15	**Aidan McKeon, Bond University** *Investigating the effects of hyperglycaemia on bladder contractility in a murine model of diabetes mellitus*	
2.20	**Nicholas Rosser, Griffith University** *Exploring Butenolides as Anti-Cancer Agents: Synthesis and Analysis of a Novel Compound Library*	
2.25	**Vineesha Veer, Bond University** *The comparison of clinical antimuscarinics on urothelium and lamina propria contractile activity*	
2.30	**Chrissy Rager, Monash University / Justus Liebig University** *A novel approach to visualize superficial wall movements in seminiferous tubules*	
2.35-2.45	**Short Break**	
	SESSION 2: Oral Presentations	**Chairs:**
2.45	**Jenane Konesan, University of Wollongong** *Non-antibiotic Alternatives For The Prevention Or Treatment Of Urothelial Damage In Urinary Tract Infection*	Sab Ventura / Lu Liu
3.00	**Srijan Shrestha, University of Adelaide** *Macroalgal phlorotannins decrease both basal and cytokine-induced angiotensin converting enzyme 2 (ACE-2) expression in human intestinal and respiratory epithelial cells*	
3.15	**Masroor Badshah, Monash University** *Age dependent effects of oxytocin on bladder contractions via nuclear trafficking of the oxytocin receptor: An insight into the treatment of overactive bladder*	
3.30-3.40	**Short Break**	
	SESSION 3: Oral Presentations	**Chairs:** Kylie Mansfield / Russ Chess-Williams
3.40	**Damian Nilsson, Bond University** *Alternative actions of phenylephrine in the porcine superior vesical artery*	
3.55	**Felix Bennett, Monash University** *Using cryo-EM to solve the P2X1 receptor structure – a target for male contraception*	
4.15-5.00	**MIXER / VIRTUAL DRINKS / PRIZE PRESENTATION** Russ Chess Williams / Betty Exintaris	

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE: Future therapies for the treatment of bladder contractile disorders?

AUTHORS: Charlotte Phelps, Russ Chess-Williams, Christian Moro

AFFILIATIONS: Centre for Urology Research, Faculty of Health Sciences and Medicine, Bond University

Strong and sustained bladder contractions are vital for voiding, however, if abnormal or spontaneous contractions occur during the filling stage, bladder dysfunction may arise. One common presentation is underactive bladder, where patients present with symptoms of urgency, weak stream, nocturia, and urinary frequency. However, there is a limited amount of research focussed on the mechanisms underlying underactive bladder, and therefore a paucity of treatment options available (1). This emphasises the need to identify novel targets in the urinary bladder that can be used in future therapies. This study aimed to determine the influence of extracellular calcium (Ca^{2+}) in G protein-coupled receptor-mediated contraction of the urinary bladder urothelium and lamina propria (U&LP). Strips of U&LP were isolated from porcine bladder and suspended in organ baths containing Krebs solution at 37°C and perfused with carbogen gas. Tissue contractions were recorded before and after the addition of a single dose of GPCR agonist in the absence and presence of 1μM nifedipine or nominally zero Ca^{2+} solution. When receptor agonists carbachol (1μM), histamine (100μM), 5-HT (100μM), NKA (300nM), PGE2 (10μM), and ATII (100nM) were added to the tissues, U&LP baseline tension increased significantly for all activated receptors ($p < 0.001$). In the presence of the L-type Ca^{2+} channel inhibitor nifedipine (1μM), or nominally zero Ca^{2+} solution, receptor-mediated contractions were inhibited. On average, Ca^{2+} influx from extracellular sources was responsible for between 20–50% of receptor-mediated contractions. Extracellular Ca^{2+} plays an essential role across many physiological functions, and mediates not only contraction, but also key Ca^{2+}-dependent systems which could be altered in bladder disorders. This study supports the suggestion of a prominent role of extracellular Ca^{2+} for urinary bladder contractile activity, presenting a mechanism potentially underlying underactive bladder.

Reference

1.Moro, C., Phelps, C., Veer, V., Clark, J., Glasziou, P., Tikkinen, K. A. O., & Scott, A. M. (2021). The effectiveness of parasympathomimetics for treating underactive bladder: A systematic review and meta-analysis. Neurourol. Urodynam. 41(1), 127-139.

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE: Investigating the effects of hyperglycaemia on bladder contractility in a murine model of diabetes mellitus

AUTHORS: Aidan McKeon, Catherine McDermott, Russ Chess-Williams, Donna Sellers.

AFFILIATIONS: Centre for Urology Research, Faculty of Health Sciences and Medicine, Bond University, QLD

Despite being extremely common in diabetic patients, complications of the lower urinary tract are much less well researched compared to the more widely known cardiovascular, retinal and renal complications. Of these complications, diabetic bladder dysfunction (DBD) is prevalent and associated with a range of symptoms ranging from bladder overactivity through to underactivity and decompensation (Panigrahy et al, 2017). However, the mechanisms underlying this progression are not clear. The aim of this study was to examine the effects of hyperglycaemia on bladder function in a mouse model of type 1 diabetes mellitus.

Female C57BL/6J mice (11-14 weeks) were administered streptozotocin (i.p. 50mg/kg) to induce diabetes, or citrate buffer for controls, daily for 5 days. At day 16 mice were euthanised, blood glucose measured using a glucometer and whole bladders isolated, cannulated via the urethra and placed in tissue baths containing Krebs-bicarbonate solution (37°C, 95%O2/5%CO2). Bladder function was assessed by measurement of spontaneous activity, bladder accommodation, and contractile responses to electrical field stimulation (EFS) and pharmacological agents.

Animals were stratified according to blood glucose levels, control <11mM (8.9±1.4mM, n=6), mild hyperglycaemia 11-17mM (MHDb, 12.9±2.0mM, n=7) and severe hyperglycaemia >20mM (SHDb, 24.6±5.9mM, n=4). Severe hyperglycaemia resulted in impaired nerve-mediated contractile responses to EFS at both 10 and 20Hz, relative to controls and mild hyperglycaemia (Fig. 1A, $P<0.05$, $P<0.01$, one way ANOVA plus Tukey's). The relative contributions of the neurotransmitters ATP, ACh and NO to the nerve-mediated responses were however unaltered. Carbachol caused concentration-dependent muscarinic-receptor-mediated contractions of bladders, and maximal responses were significantly reduced by severe hyperglycaemia compared to controls and mild hyperglycaemia (Fig. 1B, $P<0.05$). Contractions of bladders to ATP were also similarly impaired by severe hyperglycaemia (Fig 1C, $P<0.05$), whilst contractions to high KCl (60mM), spontaneous phasic activity and bladder accommodation following filling were unaltered and similar between animal groups.

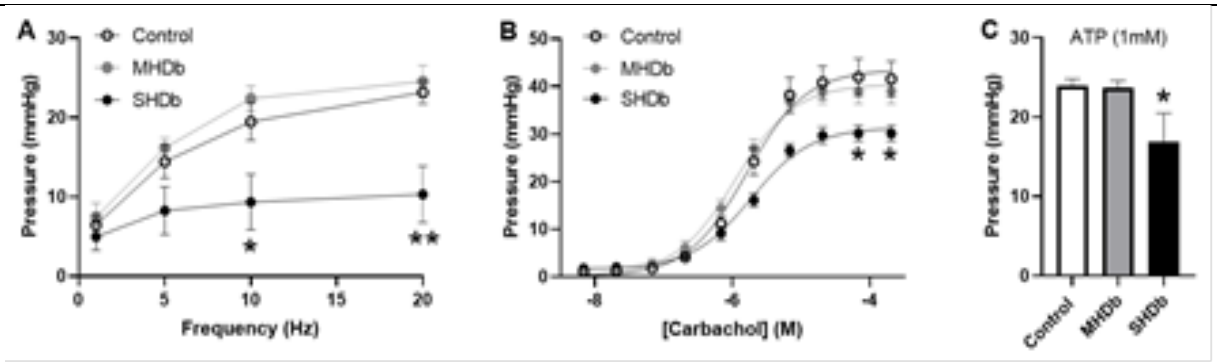

Figure 1. Contractile responses of isolated whole bladders evoked by electrical field stimulation (A), carbachol (B) and ATP (C). Data is mean ± SEM, * P<0.05, **P<0.01 vs control and mild hyperglycaemia, one way ANOVA Tukey's.

In conclusion severe hyperglycaemia impairs a number of key control mechanisms involved in bladder contraction (neuronal, cholinergic and purinergic responses) without changing overall bladder contractility, changes that were not observed with mild hyperglycaemia. These changes may partly explain the decompensation and decreased sense of bladder fullness experienced by diabetic patients.

Panigrahy R. et al. 2017 Diabetes Metab Syndr 11:81–82

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE:
Exploring Butenolides as Anti-Cancer Agents: Synthesis and Analysis of a Novel Compound Library

AUTHORS:
Nicholas Rosser, Prof Shailendra Anoopkumar-Dukie[1], Assoc Prof Milton J Kiefel[2]

AFFILIATIONS:
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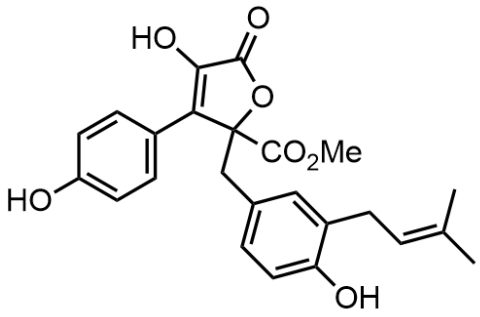
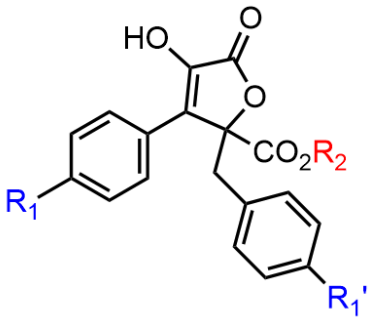
The marine fungus *Aspergillus terrus* produces a number of structurally complex molecules known commonly as Butenolides. Butyrolactone I, an archetypical butenolide, has been shown to inhibit both Cyclin-Dependant Kinase 1 and 2 (CDK1 and CDK2, respectively). Given the vital role these CDK enzymes play in regulation of the cell cycle, there is potential for these compounds to be used as anti-cancer agents in a variety of cancers.

Despite these findings, research into the use of these compounds in cancer has been limited – with their structural complexity limiting their efficient synthesis. However, a new method developed by Everson and Kiefel allows analogs of these butenolides to been made in a simple and robust 2-step Horner-Wadsworth-Emmons (HWE) reaction.



Butyrolactone I



HWE Butenolide

Using this new HWE method, we have synthesised 10 butenolides (including 8 novel compounds) with aryl substituent variations, as well as with varying exocyclic esters. The anti-cancer activity of these compounds is currently being evaluated in the PC-3, LNCaP and WPMY-1 prostate cell lines for use as potential anti-proliferative agents in prostate cancers. Our early findings have shown many of these compounds produce anti-proliferative effects, with our continuing research aiming to improve and explore this activity further.

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Advances in Urogenital and Gut Research Symposium

TITLE: The comparison of clinical antimuscarinics on urothelium and lamina propria contractile activity

AUTHORS: Vineesha Veer, Russ Chess-Williams, Christian Moro.

AFFILIATIONS: Faculty of Health Sciences and Medicine, Bond University

Introduction: Overactive bladder is the most common type of bladder dysfunction and involves spontaneous contractions of the urinary bladder during the filling phase. Antimuscarinic medications remain the first-line pharmaceutical therapy for the treatment of overactive bladder (1), with the primary action to block muscarinic receptors from the action of acetylcholine, inhibiting contractions. However, more than 70% of patients who are administered these drugs cease the treatment regimen due to lower than expected treatment benefits or adverse side effects (2). The reason for this is unclear; however, it could mean that there is varied effectiveness or selectivity of antimuscarinics on urinary bladder tissue. **Aim:** This study aims to find the differences in the abilities to inhibit contractions of the U&LP for commonly prescribed clinical antimuscarinics. **Methods:** Strips of porcine urothelium were mounted in carbogen-gassed Krebs-bicarbonate solution at 37°C. The tissues were paired with carbachol concentration-response curves performed in the absence or presence of each selective antagonist. pEC50 values for each curve were analysed and estimated affinities calculated. **Results:** The clinical antimuscarinics that produced right parallel shifts from the control (concentration; n value; estimated affinity or pkD; unpaired Student's two-tailed *t*-test) included oxybutynin (1mM; 23; 7.53; 0.00), solifenacin (1mM; 12; 6.96; 0.00), darifenacin (100nM; 12; 6.54; 0.00), tolterodine (1mM; 12; 7.96; 0.00), trospium (100nM; 12; 7.60; 0.00) and fesoterodine (100nM; 12; 7.37; 0.00). Propiverine (1mM) did not produce a shift (1mM; 12; 5.68; 0.39). **Conclusion:** This data suggests that there is a varying degree

of effectiveness for each antimuscarinic to inhibit tonic contractions of the bladder urothelium and lamina propria in response to muscarinic receptor stimulation.

References

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Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE:
A novel approach to visualize superficial wall movements in seminiferous tubules

AUTHORS:
Rager C.[1], Mietens A.[2], Ernst A.[2], Tasch S.[2], Nowell C.[1], Saikiraglou T.[2] & Middendorff R.[2]

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2) Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen

In the testis, smooth muscle cells (SMCs), also referred to as peritubular cells (PTCs), surround the germinal epithelium of the seminiferous tubules (STs). STs host the proliferation and maturation of male germ cells to become spermatozoa. Once shed from the epithelium, spermatozoa are still immotile hence require an active driving force propelling them towards the rete testis. In this regard, the contractility of PTCs plays a pivotal role for male reproduction. In rodents the spermatogenic stages are arranged section-wise and can be distinguished by histology and transillumination.

In this study a custom-built Fiji-based code has been established to characterize spontaneous superficial wall movements of two spermatogenic stages (**dark**: before and **pale**: after spermiatio) in isolated rat STs. The latter confirmed a significant difference in the spontaneous contraction patterns of dark and pale tubules.

When treating both tubule stages with a donor of nitric oxide (NO), known to relax SMCs, the code detected a significant reduction of the spontaneous contractions, independent of the respective spermatogenic stage.

In STs of human patients, spontaneous contractions and NO-induced effects could also be visualized by using the same *ex vivo* approach.

In agreement, isolated human PTCs revealed an increase of relaxation-mediating cGMP by NO and showed a reduced calcium increase by noradrenaline when inhibiting cGMP hydrolysis with sildenafil.

Different contraction patterns of the observed ST stages (before and after spermiatio) might reflect different local functions. The luminal fluid in different sections leads to a varying internal pressure on the ST wall. Since the NO-synthesis is known to be stretch-induced, modulating local NO-effects on the germinal epithelium seem likely.

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TITLE: Non-antibiotic Alternatives For The Prevention Or Treatment Of Urothelial Damage In Urinary Tract Infection

AUTHORS: Jenane Konesan

AFFILIATIONS: UNSW

Introduction: Urinary tract infections (UTI) are common infections experienced by 80% of women during their lifetime. (1) Antibiotic resistance is increasing and the identification of agents that can effectively prevent damage to the urothelium associated with UTI is essential. Cranberry, D-mannose and Ibuprofen have been tested in clinical trials for either preventing or treating UTIs in women. This study examined the effect of three non-antibiotic agents (cranberry, D-mannose and Ibuprofen) on urothelial physiology including viability and ability to form an impermeable barrier. Aims. This project involved establishing an *in vitro* model using MDCK urothelial cells and to assess the impact of urothelial integrity with UPEC and cranberry, D-mannose and Ibuprofen.

Methods: Cell viability was used to measure oxidation of resazurin over a 6-hour period. Transepithelial resistance was measured in transwell plates with UPEC, and the non-antibiotic agents to determine effect on the permeability barrier. Reactive oxidative stress production in urothelial cells was also measured with UPEC and the non-antibiotic agents.

Results: Pretreatment of UTI89 with cranberry (3 mg/ml) for 90 min significantly prevented UTI89-induced decrease in cell viability **(Figure 1)**. The microscopic images below also demonstrate this protective effect after 6 hours **(Figure 1A)**. Pretreatment of UTI89 with cranberry also partially but significantly inhibited UTI89-caused damage to monolayer barrier integrity **(Figure 2)**.

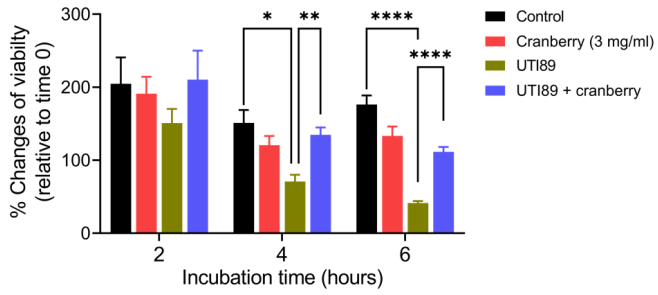

Figure 1: Change in percentage of cell viability

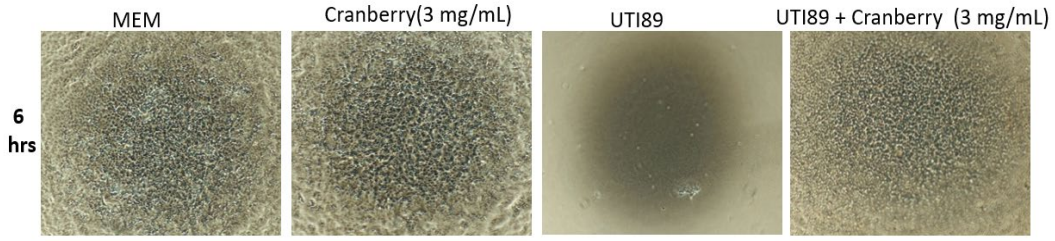

Figure 1A: Microscopic images of UTI89 with cranberry (3mg/mL) after 6 hours

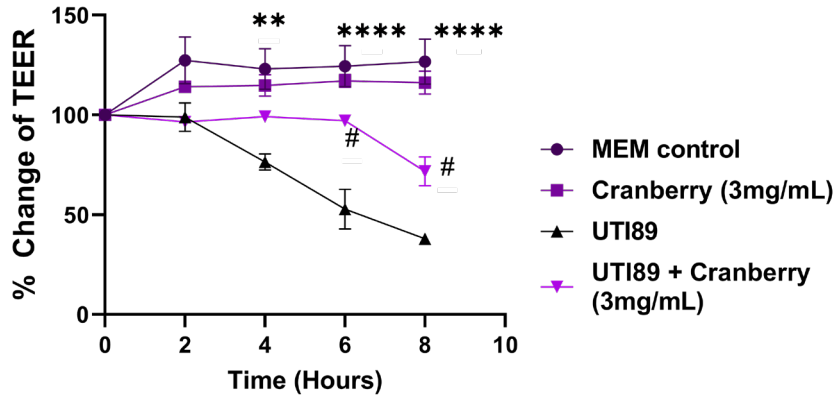

Figure 2: TEER results for cranberry (3mg/mL)

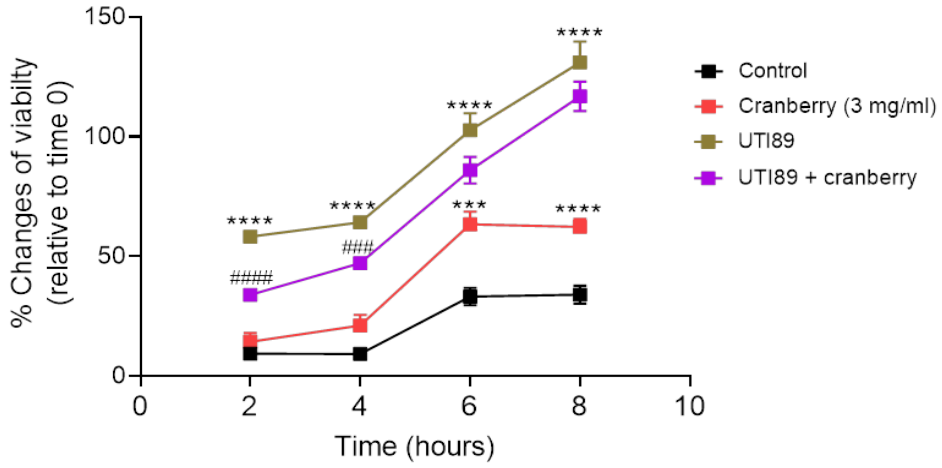

Figure 3: ROS production when cranberry (3mg/mL) was co-incubated with UTI89 for 6 hours

Cranberry increased ROS at 6 and 8 h but was partially protective at 2 h and 4 hrs **(Figure 3)**.
Discussion: *Uropathogenic E coli* UTI89 significantly reduced MDCK cell viability, TEER values and increased ROS production after co-incubation of UTI89 with cells for 4 and 6 hours. Co-incubating UTI89 with cranberry (3 mg/ml) for 90 min significantly prevented UTI89-induced decrease in cell viability. Cranberry (3 mg/m/) partially but significantly inhibited UTI89-caused damage to monolayer barrier integrity (**TEER)**. Cranberry (3 mg/ml) alone increased cell ROS production at 6 and 8 hours, but significantly inhibited UTI89-induced ROS production at 2 and 4 hours. D-mannose at 30 mM showed a toxic effect and reduced cell viability. D-mannose and ibuprofen (up to 100 µg/ml) did not show any effect in preventing UTI-induced cell damage (data not shown).

(1) Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology*, *13*(5), 269–284. https://doi.org/10.1038/nrmicro3432

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE: Macroalgal phlorotannins decrease both basal and cytokine-induced angiotensin converting enzyme 2 (ACE-2) expression in human intestinal and respiratory epithelial cells

AUTHORS: Srijan Shrestha[1], Jae Sue Choi[2], Wei Zhang[3,4], Scott D. Smid[1]

AFFILIATIONS: [1]Discipline of Pharmacology, School of Biomedicine, Faculty of Health Sciences, The University of Adelaide, South Australia, Australia
[2]Institute of Fisheries Sciences, Pukyong National University, Busan 46041, Korea
[3]Centre for Marine Bioproducts Development (CMBD) and [4]Dept. of Medical Biotechnology, College of Medicine and Public Health, Flinders University, South Australia, Australia

As angiotensin converting enzyme 2 (ACE-2) enzyme is exploited by SARS-CoV-2 to gain epithelial cell entry with subsequent viral replication and covid-19 disease pathology, ACE-2 has emerged as a potential target for the development of SARS-CoV-2 therapies. Macroalgal (seaweed) phlorotannins have been shown to exhibit a variety of biological actions and are well tolerated by humans. In the present study, the effects of two eckol-type (eckol and dieckol) and fucofuroeckol-type (phlorofucofuroeckol-A (PFFA) and 974-A) phlorotannins in modulating basal and cytokine-stimulated ACE-2 expression and activity in intestinal Caco-2 and respiratory A549 cells were investigated. Results indicated that phlorotannins were innocuous to both Caco-2 and A549 cells up to 100 μM. Proinflammatory cytokine (TNF-α and IL-1β) incubation increased ACE-2 expression in both Caco-2 and A549 cell lines. In Caco-2 cells, eckol and dieckol had negligible influence on either basal or cytokine-stimulated ACE-2 expression. In A549 cells, dieckol significantly reduced basal ACE-2 isoform A expression, while eckol and dieckol significantly reduced the cytokine-induced expression of ACE-2 isoform A. In the Caco-2 cell line, however, PFFA and 974-A significantly reduced ACE-2 expression. Additionally, both compounds downregulated the basal and cytokine-induced expression of both isoforms A and B, with the exception of isoform B in PFFA-treated A549 cells. Altered expression profiles were accompanied by substantial inhibition of cytokine-induced ACE-2 enzymatic activity in PFFA and 974-A- treated cells. Overall, our findings demonstrate that phlorotannins can variably modulate the expression and activity of ACE-2 in both basal and pro-inflammatory settings and suggest these macroalgal polyphenols may therefore inform further development of COVID-19 treatment strategies centred upon prophylaxis and virulence mitigation.

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE: Age dependent effects of oxytocin on bladder contractions via nuclear trafficking of the oxytocin receptor: An insight into the treatment of overactive bladder

AUTHORS: M. Badshah[1], J.P. Ibrahim[2], Penny AF Whiley[1], R. Middendorff[3], M.R. Whittaker[4] & B. Exintaris[5]

AFFILIATIONS: [1]Department of Molecular and Translational sciences, Monash university, Clayton, VIC, Australia
[2]School of Biomedical Sciences, University of Queensland, Queensland, Australia
[3]Institute of Anatomy and Cell Biology, Justus-Liebig-University, Giessen, Germany
[4]Drug Discovery Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia
[5]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia

Overactive bladder (OAB) is an umbrella term used to describe adverse changes in urinary frequency, urgency, nocturia and urge incontinence. While symptoms become more pronounced with age, they may occur at any age, leading to a progressive decline in quality of life. These urinary changes may be due to upregulation of oxytocin receptors (OXTRs) that are found within the detrusor muscle, resulting in an increase in myogenic tone within dynamic components of the bladder. Thus, we hypothesised that oxytocin (OT) stimulates OXTRs resulting in an increase in smooth muscle contractions within the bladder. Immunohistochemistry studies indicated significant expression of OXTR within both epithelial and stromal compartments of bladders from young (7-8 weeks) and old (16 weeks) Sprague - Dawley male rats (n=5/group). Interestingly, this OXTR staining was predominantly localised to the nucleus within both age groups (unpaired t-test, $p < 0.05$). Therefore, oxytocin may interact via nuclear specific OXTR resulting in a substantial increase in bladder contraction within both young and older rats. This study supports our previous organ bath findings that OT is modulator of bladder contractility. Further studies are needed to determine the physiological pathways of OXTRs within the bladder to improve our understanding of OAB and lower urinary tract symptoms (LUTS), and to explore future therapeutic opportunities.

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE:
Alternative actions of phenylephrine on the porcine superior vesical artery

AUTHORS: Damian Nilsson, Donna Sellers, Russ Chess-Williams

AFFILIATIONS:
Centre for Urology Research, Bond University, QLD

Introduction
In functional pharmacological investigations, phenylephrine has been frequently used as an α_1-adrenoceptor agonist. However, it has previously been demonstrated to mediate responses via β-adrenoceptor stimulation (Chess-Williams, et al. 1990). It has also been demonstrated to increase the potency of responses to 5-hydroxytryptamine (5-HT) (Movahedi, et al. 1995; Chen, et al. 2000). The aim of this study was to determine whether phenylephrine mediates action only via α_1-adrenoceptors.

Methods
Superior vesical artery (SVA) branches from pigs (6-month old) were obtained from a local abattoir. Sections (~4mm length, ~1mm internal diameter) were isolated and mounted between two horizontal stirrups in 10mL organ baths containing oxygenated physiological salt solution at 37˚C. Concentration-response curves to phenylephrine and 5-HT on arterial rings. Phenylephrine responses were performed in the presence of prazosin, tamsulosin, ketanserin, idazoxan, yohimbine, αβmATP, propranolol, L-NNA, indomethacin, corticosterone and desipramine. 5-HT responses were performed in the presence of prazosin and ketanserin.

Results
Phenylephrine caused dose dependent vasoconstrictions to porcine superior vesical artery rings, producing a biphasic response. After reaching the initial maximum (5μM) of the responses (Figure 1A), continued additions of phenylephrine caused relaxation of the tissues (Figure 1A). However, at concentrations of ~50μM and above, the arterial rings resumed contraction once more. A true maximum response could not be research due to the high concentrations of phenylephrine required. This biphasic response was not observed to A-61603 and noradrenaline. In the presence of α_1-adrenoceptor antagonists, the first phase of the curve was shifted rightwards. The second phase was unaffected. The biphasic curve was unaffected by nitric oxide synthase, ATP, cyclooxygenase, uptake 1 and 2 inhibitors and imidazoline, β and α_2-adrenoceptor antagonists. Ketanserin significantly affected the phenylephrine biphasic responses (Figure 2), suggesting the component is via 5-HT receptors. 5-HT is a potent vasoconstrictor on the porcine SVA (Figure 1B), antagonised by ketanserin, but not prazosin (Figure 1C and 1D).

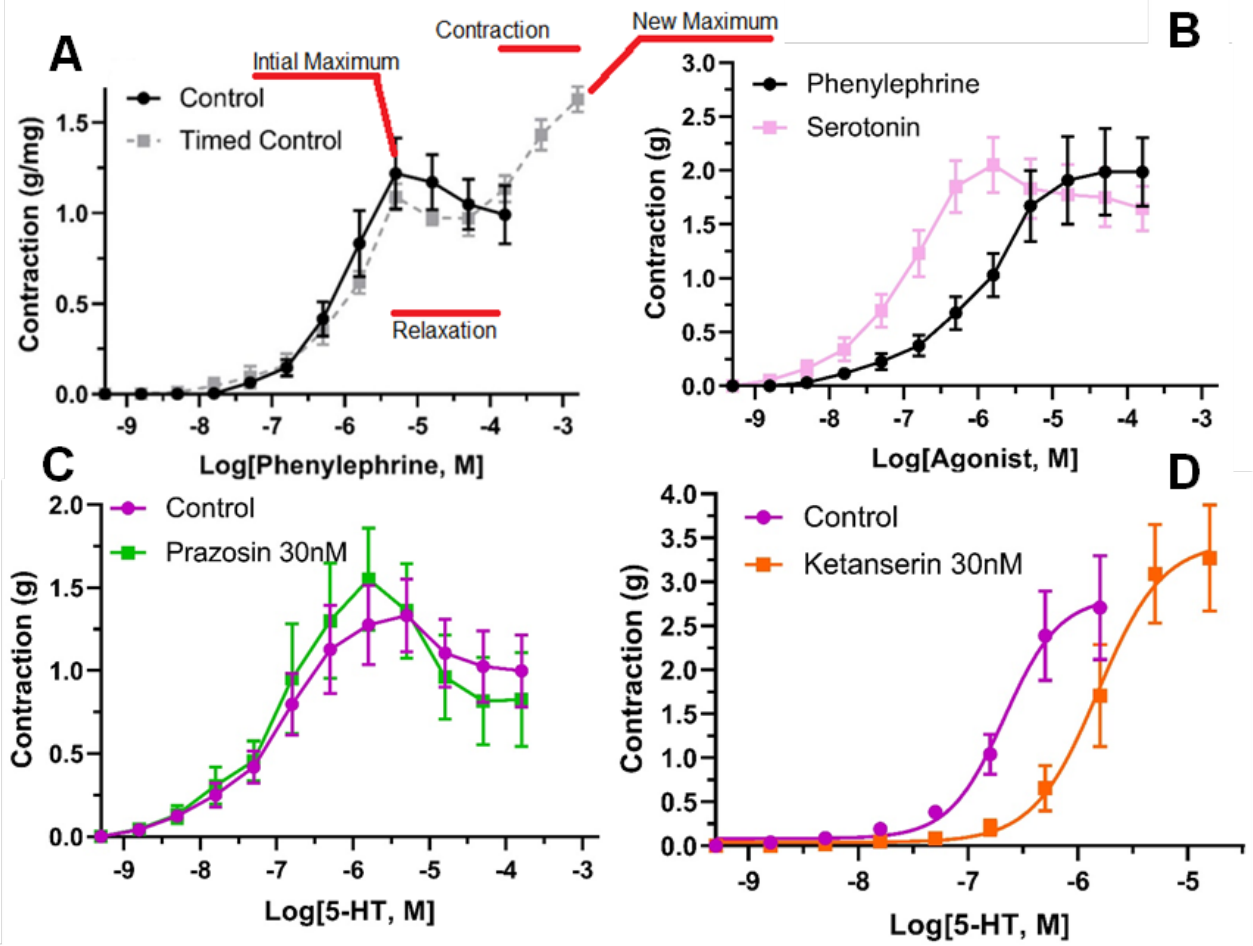

Figure 1: Agonist responses on the porcine superior vesical artery. A) Repeating responses to phenylephrine, demonstrating biphasic responses. B) Responses to 5-HT and phenylephrine. C) Responses to 5-HT in the presence of α_1-antagonist prazosin. D) Responses to 5-HT in the presence of 5-HT antagonist ketanserin. Data is mean ± S.E.M, normalized to mass (g/mg).

Conclusion

The initial contraction response of the porcine SVA to low concentrations of phenylephrine was mediated by α_1-adrenoceptors. At higher concentrations, phenylephrine produces a relaxation followed by a further contraction which were mediated via 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors, respectively.

References

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Movahedi, H., Le, H. T., Spanggord, H. M., & Purdy, R. E. (1995). J Pharmacol Exp Ther, 272, 364-370.

Virtual National Symposium

Advances in Urogenital and Gut Research Symposium

TITLE: Using cryo-EM to solve the P2X1 receptor structure – a target for male contraception

AUTHORS: Felix M Bennetts[1], Jesse I Mobbs[1,3], Alisa Glukhova[2,3], David M Thal[1,3] & Sabatino Ventura[1]

AFFILIATIONS: Drug Discovery Biology Theme[1], Monash Institute of Pharmaceutical Sciences, Melbourne, VIC, Australia; Walter and Eliza Hall Institute of Medical Research[2], Melbourne, VIC, Australia; ARC Centre for Cryo-electron Microscopy of Membrane Proteins[3], Monash Institute of Pharmaceutical Science, Melbourne, VIC, Australia

331,000 unintended pregnancies are conceived every day yet, there are only two male contraceptives available. A novel target for male contraception is the P2X1 receptor which is genetically validated but further studies are held back by a lack potent P2X1 receptor antagonists. To accelerate drug discovery efforts at the P2X1 receptor this project aims to solve a high-resolution structure of the P2X1 receptor and use this information to guide the design of highly potent P2X1 receptor antagonists. Full-length human P2X1 receptor was purified using a membrane purification preparation. Cryogenic transmission electron microscopy (cryo-EM) was used to solve the P2X1 receptor structure. P2X1 receptor antagonists were validated using a HEK293 P2X1 expressing cell line in an intracellular calcium mobilisation assay and a radioligand binding assay. Initial cryo-EM images of the P2X1 receptor revealed severe preferred orientation of the receptor in vitreous ice. The addition of a secondary detergent, fluorinated FOS-Choline-8, significantly reduced preferred orientation which assisted in obtaining a 1.96 Å structure of the P2X1 receptor in an ATP bound state. The activity of P2X1 receptor antagonists were validated in pharmacology assays. The next step is to generate novel and more effective P2X1 receptor antagonists leveraging our high-resolution P2X1 receptor structure and optimised cryo-EM workflow.

1.96 Å ATP-Bound P2X1 Model