Drug targets for urinary and faecal incontinence and anal fissures

PhD Thesis

By

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Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

September 2016
ABSTRACT

As life expectancy increases due to better diagnosis and treatments for chronic diseases, the ageing population and the number of people suffering from incontinence will increase. Thus, although non-life threatening, there is a need for better treatments for these conditions.

Correct functioning of the urethra and the anorectum is reliant on the smooth muscle of these tissues. The internal anal sphincter of the anorectum provides most of the resting anal pressure, while the urethral circular smooth muscle provides the resting urethral pressure. Loss of tone in these smooth muscles is associated with faecal incontinence and stress urinary incontinence respectively. Presently, there are no effective pharmacological treatments for stress urinary incontinence or faecal incontinence. Adverse side effects have hampered the development and use of pharmacotherapies for these conditions, mainly due to a poor understanding of urethral and anorectal function.

The aim of this PhD project was to increase our understanding of the pharmacology of the urethra and the anorectum. Specifically, the intent was to elucidate the pharmacology of the smooth muscle of the urethra and the anorectum; to establish the influence of the urothelial lining on urethral contraction; to investigate the mediators and neurotransmitters involved in the regulation of these tissues and to characterise the intracellular signalling pathways involved in contraction and relaxation.

Regional differences, age-related differences and signalling pathways in response to G-protein-coupled receptor activation were investigated directly in the urethra, using an in vitro organ bath technique with isolated porcine tissues (Large white-Landrace), whilst the neurotransmitters of the internal anal sphincter were investigated using electrically field stimulated porcine tissues.

Using these techniques, a role for cellular Ca\(^{2+}\), protein kinase C and Rho kinase in influencing the basal tone and receptor-mediated responses of the urethra was investigated. Experiments investigating urethral \(\alpha_1\)-adrenoceptor-mediated responses showed the proximal urethra to be the region with the greatest contractile response, and this was similar in tissues from young (6 months) and older (36 months) pigs. The urothelium/lamina propria of the
urethra had an inhibitory effect on the underlying smooth muscle that did not involve prostaglandins or nitric oxide.

Ca\textsuperscript{2+}-sensitization which is the increase in Ca\textsuperscript{2+}-sensitivity of the contractile apparatus, prostaglandins and Ca\textsuperscript{2+} influx via L-type Ca\textsuperscript{2+} channels all contributed to the basal tone developed by the urethra. Likewise, pharmacological inhibition experiments showed that the potency of the α\textsubscript{1}-adrenoceptor agonists was proportional to their ability to induce Ca\textsuperscript{2+} sensitisation. A61603, a specific α\textsubscript{1A}- adrenoceptor agonist, produced a long-lasting contractile tone, whilst tone was not maintained following stimulation with noradrenaline.

Incubation of tissues with A61603 and phenylephrine caused desensitisation of subsequent responses to noradrenaline. Rho kinase and protein kinase C activity, as well as cytoplasmic Ca\textsuperscript{2+}, contributed to the A61603-induced desensitisation, whilst only Rho kinase contributed to phenylephrine-induced desensitisation. These results suggest Rho kinase involvement in a feedback mechanism to prevent chronic overstimulation of the α\textsubscript{1}-adrenoceptor in the urethra. Prior activation of muscarinic receptors before activation of α\textsubscript{1}-adrenoceptors had no significant effect on α\textsubscript{1}-adrenoceptor-mediated responses in the presence of the urothelium/lamina propria. However, in the absence of the urothelium/lamina propria, prior activation of muscarinic receptors significantly reduced the α\textsubscript{1}-adrenoceptor-mediated responses, suggesting the modulation of receptor interactions by the urothelium/lamina propria. This interaction was independent of neuronally released factors or prostaglandins.

Finally, this study showed co-transmission in the internal anal sphincter, with neurogenic contraction mediated by noradrenaline and ATP, whilst neurogenic relaxation of the porcine internal anal sphincter was mediated by the simultaneous release of the gasotransmitters nitric oxide, carbon monoxide and hydrogen sulphide, with relative contributions of nitric oxide > carbon monoxide > hydrogen sulphide. Neurotransmission in the pig internal anal sphincter does not appear to involve acetylcholine. All of these transmitters represent possible targets for drug development, where enhancing sphincter tone may aid the treatment of faecal incontinence or reductions in internal anal sphincter tone may aid healing of anal fissures.

Based on these observations Ca\textsuperscript{2+} sensitization is a significant pathway involved in the mediation of urethral contractile responses and basal tone, as well as modulation of α\textsubscript{1A}-
adrenoceptor desensitisation. Thus, the Ca$^{2+}$ sensitization pathway stands out as a prospective drug target for the development of new treatments for lower urinary tract symptoms such as stress urinary incontinence.

Further research is necessary to determine the relative contribution of the Ca$^{2+}$ sensitization pathways in incontinence, as there is the possibility of a switch towards a reduced Ca$^{2+}$ sensitization mechanism, which could underlie stress urinary incontinence and faecal incontinence, whilst greater Ca$^{2+}$ sensitization may underlie hypertonia of the internal anal sphincter resulting in an inhibition of healing of anal fissures.
DECLARATION OF ADDENDUM

‘The work presented in this document has not been submitted for a degree or any other purpose at this university or any other institution. To the best of my knowledge, this report has no material already published or written by any other person except where due reference is made’

Signed:

Oladayo Seun Folasire

Date: September, 2016.
ACKNOWLEDGEMENT

I would like to thank my wife, Esther, for her support, understanding, encouragement and motivational speeches. No one and nothing in this world is perfect, but the two of us are. To Diadem, I do appreciate your little understanding that I need to work late sometimes and might not have the time to play as much as you would like. Thank you all for tolerating my countless project papers all over the places. Likewise, I appreciate my mum, Alice, for her support. She deliberately left Nigeria to lend a hand of support during the challenging time I needed her most. Taking care of the boy was a great relief with your presence. I could not have achieved this without your support.

To my supervisors Associate Professor Donna Sellers and Professor Russ Chess-Williams, thank you for your tolerance, support, guidance and assistance from the beginning of the project. You have improved my scientific writing abilities, and I am extremely grateful for your expert support.

I thank the Centre for Urology Research, Bond University, your contribution and scientific advice counts a lot. And to my fellow PhD students, you are a great motivation to me. When I see you go through the same ordeal during the process, I feel motivated and stronger with the feeling that I am not alone.

I also appreciate the support of the administering staff of Bond University Faculty of Health Sciences and Medicine and Bond University Office of Research Services.

Finally to my siblings, friends, in-laws, uncles, especially Chief T.A Fadairo for his impact on my academic life. Your contribution since the beginning has resulted in a bigger result. Likewise, I cannot drop my pen without say thank you to the Alpha and Omega, the author and finisher. His contribution, support and encouragement surpassed my thinking.

Thank you.
Abbreviations

AC: Adenylate cyclase
AMP: Adenosine monophosphate
AP-2: Adaptor protein complex 2
ATP: Adenosine triphosphate
BK: Bradykinin
BOO: Bladder outlet obstruction
CD117: Cluster of differentiation 117 protein
CO: Carbon monoxide
CPI-17: PKC-potentiated inhibitory protein for protein phosphatase-1 of 17KDa
CREB: Cyclic AMP-response element binding protein
CRELD1: Cysteine Rich With EGF Like Domains 1
EFS: Electrical field stimulation
ET: Endothelin
GMP: Guanosine monophosphate
GPCR: G-protein-coupled receptor
GRK: G-protein-coupled receptor kinase
H_2S: Hydrogen sulphide
IAS: Internal anal sphincter
ICC -Interstitial cells of Cajal
ICC-LCs -Interstitial cells of Cajal -like cells
LP: Lamina propria
mACHr: Muscarinic acetylcholine receptor
MAPK: Mitogen-activated protein kinase
MYPT1: Myosin phosphatase target subunit 1
NO: Nitric oxide
NOS: Nitric oxide synthase
OAB: Overactive bladder
ODQ: 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one
PG: Prostaglandins
PI3K: Phosphoinositide-3-kinase
QOL: Quality of life
RhoA: Ras homolog gene family, member A
ROCK: Rho-associated coiled-coil forming protein serine/threonine kinase (Rho kinase)
SEM: Standard error of mean
SUI: Stress urinary incontinence
TEA: Tetraethylammonium chloride
VIP: Vasoactive intestinal peptide

**Abstracts**

- Donna Sellers, **Oladayo S Folasire**, Russ Chess-Williams. Characterisation of contractile responses to $\alpha_1$ adrenoceptor agonists in the porcine urethral circular smooth muscle. Abstract presented at ASCEPT-MPGPCR 2013 Joint Scientific

Presentations

- Oladayo S. Folasire, Donna J. Sellers & Russ Chess-Williams; Characterisation of contractile responses to $\alpha_1$ adrenoceptor agonists in the porcine urethral circular smooth muscle. Presented at 5th National Symposium on Advances in Gastrointestinal and Urogenital Research, 2013.
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