7

Nile C[1], Van Koeveringe G[2], Gillespie J[1]
1. *Newcastle University, UK,* ***2.*** *University Hospital Maastricht, The Netherlands*

COMPLEX SIGNAL INTERACTIONS INVOLVING ACETYLCHOLINE, MUSCARINIC TYPE 2 RECEPTORS, ATP AND PROSTAGLANDINS IN THE LAMINA PROPRIA: A 'DANGEROUS' HYPOTHESIS

Hypothesis / aims of study

The urothelium releases acetylcholine (Ach), ATP, Nitric Oxide (NO) and prostaglandin (PG). The physiological role of these signals is poorly understood and a possible interaction between them has not been considered. The aim of this study was to characterise the cholinergic system. The presence of both M_2 and M_3 muscarinc receptors in the lamina propria is not in doubt. The receptors are found in high abundance (M_2>M_3) but their physiological roles are poorly understood. One idea is that acetylcholine is released when the bladder is stretched and activates receptors on afferent nerve fibres to affect sensation [1]. However muscarinic receptors are found on other cell types e.g. sub-urothelial interstitial cells [2]. These cells also express a number of physiological and immunological receptors and appear to be a point of signal integration in this region of the bladder wall. It has recently been shown that ATP can regulate PG release from a preparation of the isolated lamina propria [3]. This preparation allows the possibility to explore other signal interactions in the lamina propria. The present study uses the isolated lamina propria preparation to study the effects of muscarininc stimulation on prostaglandin signalling. The predominant urothelial signals, ATP, PGE_2, NO and ACh are associated in many systems with early inflammation. The output of urothelial signals has been found to occur through a number of complex interactions. The modulated urothelial output is targeted at the sub-urothelial interstitial cells which are known to express an array of cytokines. Therefore, we explored the idea that ATP, PGE_2, NO and ACh regulate levels of expression of early inflammatory cytokines IL-1 and TNF-

Study design, materials and methods

Female Dunkin Hartley Guinea pigs (200–400 g) were used. All procedures were carried out in accordance with EC guidelines on animal welfare. To investigate the role of ACh on PGE_2 production the isolated lamina propria model was used as described previously [3]. Briefly, strips of lamina propria were incubated with varying concentrations of arecaidine and the bathing media collected for analysis of PGE_2 content. In addition, similar experiments were performed in the presence of varying concentrations of muscarinic antagonists darifenacin (M_3>>M_2) and AFDX 116 (M_2>M_3). The model was also used to investigate the expression of the muscarinic receptors and early inflammatory response cytokines.

Results

Figure 1A shows the dose dependant release of PGE_2 from the lamina propria in response to the muscarinc agonist arecaidine: the half maximal concentration was 80 nM. The arecadine dependant PGE_2 release was affected by AFDX-116 (Figure 1B), with a half maximal inhibitory concentration of 200 nM. Darifenacin, had little effect, with the estimated half maximal inhibitory concentration being 2 µM (Figure 1 C). In response to exposure to muscarinic agonist and PGE_2 it was found that the levels of expression of both the M_2 and M_3 receptor mRNA changed: PGE_2 increased expression while acrecaidine decreased expression (Figure 1D). Examination of the effects of PGE_2, ATP, arecaidine and NO on early cytokine mRNA expression showed that only PGE_2 and arecaidine affected expression (Figure 2)

Interpretation of results

In the isolated bladder lamina propria ACh has a role in the regulation of PGE_2 production. The fact that AFDX-116 abolished the cholinergic induced PGE_2 release demonstrates a clear role for M_2 receptors in this phenomenon. This is the first report of a functional role for M_2 receptors in the bladder lamina propria. Interestingly, upon prolonged exposure to ACh there is a down regulation of the expression of both the M_2 and M_3 receptor mRNA. This suggests the possibility of complex regulation of the signalling system by controlling receptor expression. In an extension of this signalling system both arecaidine and PGE_2 increased the expression of early pro-inflammatory cytokines almost certainly located in the network of sub-urothelial interstitial cells. The physiological role of this lamina propria signalling system is not known. However, as many of the signals involved are associated with early inflammatory responses, it is possible that it is involved in detecting and regulating early responses to bladder damage.

Concluding message

For the first time a functional role for the M_2 receptors of the bladder urothelium has been demonstrated. Furthermore, a 'new' system has been identified which might be involved in detecting 'danger' in the bladder and triggering early inflammatory responses.

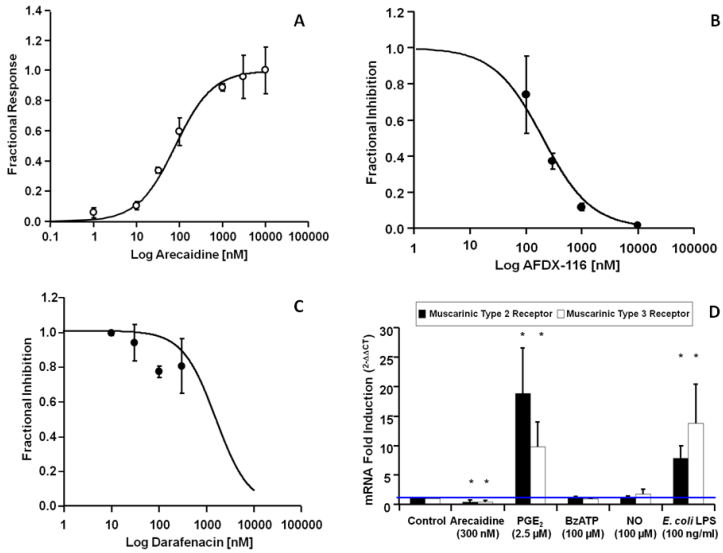

Figure 1 Responses in the isolated lamina propria preparation of the guinea pig bladder. A shows the dose dependent output of PGE_2 in response to the muscarinic agonst arecaidine. B and C show respectively the inhibitory actions of the M_2>M_3 antagonist AFDX-116 and the M_3>>M_2 antagonist darifenacin. D shows data from a quantitative analysis of the mRNA levels of M_2 and M_3 receptors in the lamina propria exposed to different stimuli (*E.coli*, LPS, PGE_2, arecaidine, BzATP and NO). Ordinate shows 'fold' increase over basal levels.

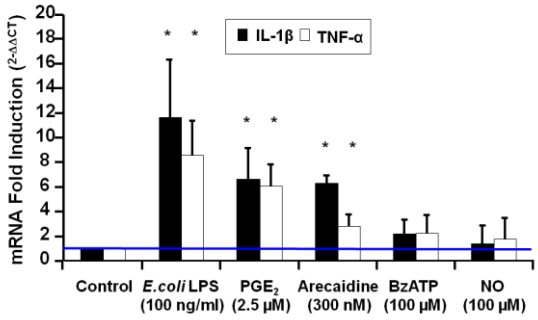

Figure 2 Data from a quantitative analysis of the mRNA levels of IL-1 and TNF in the lamna propria exposed to different stimuli (E.coli LPS, PGE_2, arecaidine, BzATP and NO). Ordinate shows 'fold' increase over basal levels.

References

1. Birder L. Role of the urothelium in bladder function. Scand J Urol Nephrol Suppl. 2004;(215):48-53.
2. Grol S, Essers PB, van Koeveringe GA, Martinez-Martinez P, de Vente J, Gillespie JI. M3 muscarinic receptor expression on sub-urothelial interstitial cells. BJU Int. 2009 Aug;104(3):398-405.
3. Nile CJ, de Vente J, Gillespie JI. Stretch independent regulation of prostaglandin E(2) production within the isolated guinea-pig lamina propria. BJU Int. 2010 Feb;105(4):540-8.

Specify source of funding or grant	**Aspects of this work were supported by the BJU International Collaborative Research award 2007 to JI Gillespie and GA van Koeveringe.**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**All procedures were approved by the institutional animal care and use committee of Newcastle University and in line with the EC guidelines.**