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Wu C[1], Sui G[1], Matharu R[1], Fry C[1], Montgomery B[2], Young J[1]
1. *University of Surrey,* ***2.*** *Frimley Park Hospital*

MUSCARINIC MODULATION OF UROTHELIAL ATP RELEASE AND ITS PARACRINE ACTION

Hypothesis / aims of study

Acetycholine and ATP are released from the urothelium and other cellular compartments in response to mechanical stretch or chemical signals[1, 2]. These transmittters may exert further actions on the afferent input and smooth muscle and hence provide a complex control over bladder function. However, the relationship between muscarinic activation and ATP release has never been explored in the physiological context and little is known about the mechanisms involved. This study used native guinea-pig urothelial sheet as a functional unit to test the hypothesis that muscarinic receptors control urothelial ATP release, and furthermore ATP may exert a paracrine effect on underlying smooth muscle.

Study design, materials and methods

Urothelial sheets and urothelium-attached detrusor muscle preparations were isolated from guinea-pig urinary bladders (male, Dunkin-Hartley, either 2-3 months or 16-18 months) by blunt dissection. Urothelial cells were freshly dissociated from the urothelial sheets using a collagenase-based enzyme mixture[3]. Human bladder mucosal samples were obtained at rigid cystoscopy. All preparations were superfused in a HEPES-buffered physiological saline, at pH7.4, 37°C and tied to an isometric force transducer to record spontaneous contractions. Intracellular Ca^{2+} was measured in urothelial cells loaded with the Ca^{2+}-sensitive fluorescent indicator Fura-2 AM using epifluorescence microscopy. The concentration of ATP was measured by sampling the superfusate adjacent to the urothelium and using a luciferin-luciferase assay. Data are expressed as mean±S.E.M. Student's t-tests were used to examine paired and non-paired normally distributed data sets, non-parametric equivalent tests were used for data sets of unknown distribution; ANOVA and a Bonferroni post-hoc test were employed for multiple comparisons.

Results

Significant release of ATP was detected from unstimulated urothelial sheets from guinea-pig bladders (563±78 pmoles/g tissue, n=46 bladders) and was increased in ageing animals (2449±798 pmoles/g tissue, n=27, $p<0.05$). ATP release exhibited spontaneous rises in one-third bladders, with an average of two-fold changes in ATP levels. Spontaneous Ca^{2+} waves were also consistently observed from isolated urothelial cells.

The cholinergic agonist carbachol (50µM) elicited a variable release of ATP from the urothelium with an overall rise (147±13 % of control, n=27, $P< 0.01$). The effect was also demonstrated with the muscarinic agonist oxotremorine (979±515 % of control, n=11, $p<0.01$) which has the highest affinity for M2 receptors. This enhancement by muscarinic agonists was diminished by atropine and methoctramine, the latter with preferential action on M2 receptors. Release of ATP from the urothelium was also triggered by physical stretch (20 % of original length). The stretch-induced ATP release was attenuated by application of the M3 antagonist 4-DAMP (297.8 ± 91 $pM.mg^{-1}$ prior to stretch and 222.9 ± 89 $pM.mg^{-1}$ immediately afterwards; a 25% decrease, n=5, $p<0.05$).

Small spontaneous contractions were observed in about one-third of urothelial sheets. Contractions could also be generated by carbachol in some urothelial sheets. The contractile activity of the urothelium was accompanied by increased urothelial ATP release.

Spontaneous contractions were also observed in urothelial-attached detrusor muscle preparations, which were usually absent in urothelium-denuded muscle preparations. This activity was enhanced by a sub-threshold concentration of carbachol (50nM, peak contraction 172±23% of control, n=7, $p<0.05$) that had a minimal effect in denuded detrusor muscle strips. The augmentation by carbachol was attenuated by desensitising the P2X receptor in the detrusor muscle with alpha, beta-methylene ATP (ABMA, a mean reduction of 59±6%, n=6, $p<0.01$)).

In human bladder mucosa biopsies, a significant intrinsic release of ATP was also observed. Carbachol evoked significant ATP release in the urothelium (566±145% of control, n=6, $p<0.05$). Carbachol-induced contractions could also be observed in some biopsies.

Interpretation of results

Urothelium exhibited active release of ATP, which was up-regulated in the ageing guinea-pig bladder. Spontaneous surges of ATP release from the urothelium may be associated with Ca^{2+} oscillations in urothelial cells.

The increase of ATP release generated by carbachol and oxotremorine suggests that musacrinic neurotransmitters can modulate ATP release, but the variable effect suggests a complex mode of action. The effect of oxotremorine and methoctramine supports that the M2 receptors are most probably involved. In contrast, the attenuation of stretch-induced ATP release by 4-DAMP demonstrates that M3 receptors are involved in this mode of ATP release. Furthermore spontaneous contractile activity was demonstrated in urothelial sheets as well as ones evoked by muscarinic agonists. The association between urothelial contractions and ATP release suggests an interaction between these two phenomena.

Urothelium-dependent spontaneous muscle contractions were augmented by subthreshold concentrations of carbachol. Attenuation of this effect by desensitising smooth muscle P2X receptors with ABMA, further suggests involvement of a paracrine inotropic effect via ATP release from the urothelium via muscarinic activation.

Urothelial ATP release and contractile activity and their response to muscarinic agonists can be demonstrated in human preparations.

Concluding message

Native urothelium behaves as a functional unit with the following characteristics 1. Actively releases ATP, a process which may be altered during ageing. 2. Muscarinic neurotransmitters exert significant control over both agonist-induced and stretch-evoked ATP release from the urothelium with differential roles for M2 and M3 receptors. 3. Urothelium has some intrinsic contractile activity which can be modulated by muscarinic agonists. 4. Urothelium contractile activity and intracellular Ca^{2+} oscillations are associated with ATP release. 5. Released ATP from the urothelium by muscarinic activation can produce a paracrine effect on intrinsic smooth muscle activity. These properties of urothelium implicate a complex control over both sensory and motor elements in the bladder wall.

References

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Specify source of funding or grant	**BBSRC** **Pfizer** **EU FP7 INComb**
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	**UK Home Office license and University of Surrey Ethics Committee Approval.** **Additional information: The study also used human bladder mucosa biopsies with the approval from Surrey Research Ethics Committee and informed patient consent and DECLARATION OF HELSINKI was followed.**