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THE REGULATION OF STRETCH-INDUCED ATP RELEASE FROM UROTHELIUM BY ADENOSINE

Hypothesis / aims of study

ATP release from the urothelium is proposed as a central step in the sensation of bladder fullness, as the nucleotide is released following stretch of the urothelium. Furthermore, ATP release is enhanced in tissue from bladders displaying heightened sensations on filling thus suggesting a pathophysiological basis of this condition. It is known that pathways mediating ion transport across the urothlium are also associated with ATP release however the particular cellular pathways that regulate ATP release are unknown. ATP itself is rapidly degraded by local ectonuclotidases and it is possible that its breakdown products exert a feedback contol in ATP release. We hypothesise that the terminal product of ATP breakdown, adenosine, exerts a depressant effect on ATP release from urothelium subjected to stretch. The study aimed to generate data to test this hypothesis, to determine which receptors may mediate any effect and if changes to intracellular Ca^{2+} play a role.

Study design, materials and methods

The study used urothelium sheets and isolated urothelial cells from rabbit and guinea-pig bladders. Urothelium sheets were mounted in Ussing chambers and stretched by removal of fluid from the serosal-facing chamber. Urothelial cells (<20 µm diameter) were isolated by collagenase-disruption of urothlium sheets. Samples of fluid close to the serosal membrane face were removed and assayed for ATP by a luciferin-luciferase assay. Adensoine was assayed from similar samples using mass spectrometry. Intracellular [Ca^{2+}] was measured using Fura-2 epifluorescence microscopy. Transurothelial potential (TEP) was measured with KCl-agar bridges on either side of the membrane. Short-circuit current, as an estimate of ion transport rate, was the current required to clamp the TEP to zero mV. ATP data are mean±SE of percentage changes from pre-intervention baseline. Comparison between data sets used paired, non-parametric Wilcoxon signed rank tests; the null hypothesis was rejected when $p<0.05$. ANOVA was used to analyse absolute values. Power calculation from previous experiments indicated that n=6-8 repeats could record a 30% change of the primary variable with 80% power.

Results

Hydrostatic stretch increased ATP release from the serosal side of the urothelium which reached a steady-state after about two minutes. Effects of subsequent interventions were analysed at three minutes after stretch. Adenosine (1-2 µM) reduced stretch-induced ATP release and abolished it completely at 10 µM. Stretch also increased adenosine levels in the serosal chamber and levels were rendered undetectable by adenosine deaminase. The action of adenosine was mirrored by two A1-receptor analogs (CPX and DPCPX, 1 µM) but the A2-receptor agonist DMPX (1 µM) had no effect. By contrast, adenosine deaminase increased stretch-induced ATP release. Adenosine and the non-specfic A-receptor agonist, NECA, generated very small and inconsistent rises of the intracellular [Ca^{2+}]. ATP itself has been reported to stimulate further urothelial ATP release; the non-nucleotide P2X3/P2X2/3 receptor antagonist A-317491 (10 µM) reduced the stretch-induced component of ATP release to 22±10% of control. ATP (10-30 µM) generated large (130 – 680 nM [Ca^{2+}]). Stretch-induced ATP release was also abolished when TEP was clamped to zero mV. A link between ATP release via the adensoine-receptor and TEP pathways was sought. Adenosine deaminase and DPCPX both increased stretch-induced increase of short-circuit current, there was no effect of DMPX. Altering TEP in the absence of stretch also increased ATP release, and effect that was abolished by adenosine (1 µM) and augmented by CPX (1 µM).

Interpretation of results

We have confirmed that stretch of the urothelium releases ATP from the serosal surface. Furthermore, increase of the transurothelial potential, itself generated by ion flux across the urothelium, also augmented ATP release. Adenosine, acting via A1 receptors, depressed ATP release and suggests it exerts a negative feedback control over ATP release. It remains to be determined if changes to the A1 receptor profile underlie increased stretch-induced ATP release in pathological bladders. The action of adensoine was not via augmentation of intracellular Ca^{2+} levels. ATP itself acted as a positive feedback regulator of urothelial ATP release. A P2X receptor antagonist reduced ATP release and ATP itself generated large intracellular Ca^{2+} transients. The actions of adenosine receptor modulators were mirrored by changes to transepithelial potential and indeed alteration of TEP itself altered ATP release. The modulation of TEP-induced ATP release by adenosine receptor ligands suggests that adenosine, through activation of A1 receptors, reduces ATP release by attenuating transepithelial ion transport.

Concluding message

ATP and its breakdown product adenosine exert opposing effects on ATP generation during urothelial stretch, and by extrapolation bladder filling. The net effect will depend upon the relative magnitude of the negative (adenosine) and positive (ATP) feedback pathways and it may be postulated that different degrees of sensations during bladder filling may represent the relative significance of these two effectors. The interdependence of adenosine and TEP-dependent modulation of ATP release suggests a final common pathway of ion transport across the urotheluim to regulate ATP release.

Specify source of funding or grant	**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	**Home Office approval for animal experiments**