The regulation of stretch-induced ATP release from urothelium by adenosine

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Introduction. Stretch-induced ATP release from urothelium is proposed as a central step in the sensation of bladder fullness. Furthermore, such release is enhanced from bladders displaying heightened responses on filling (painful bladder syndrome and detrusor overactivity) and associated with pathways mediating urothelial ion transport. ATP is rapidly broken down by local ectonucleotidases to ADP, AMP and adenosine and it is possible that these products could exert a feedback control over ATP release itself, by analogy to peripheral nerves. We hypothesised that adenosine exerts a negative feedback control over stretch-induced ATP release by a receptor-mediated process.

Methods. We used urothelium sheets dissected from rabbit bladders and mounted in Ussing chambers. Preparations were stretched by altering the transmural hydrostatic pressure gradient to generate a physical lateral stretch, observed by bowing of the preparation in the chamber. Fluid samples were aspirated from the Ussing chamber facing the basolateral (serosal) face and assayed for ATP using a luciferin-luciferase assay. Transepithelial potential (TEP) and the short-circuit current (SCC) necessary to clamp TEP to 0 mV were also recorded. SCC was used as a measure of the magnitude of transurothelial ion transport. Interventions were made to the basolateral-facing membrane. Data are mean±SD, data sets were compared using Wilcoxon signed rank tests; the null hypothesis was rejected when *p<0.05

Results. Transmural pressure (TP) gradient and ATP release; role of ATP receptor antagonist. Fig 1 shows that generating a TP gradient increased ATP release which peaked after about two minutes (left). Addition of the P2X3/P2X2/3-selective-antagonist A-317491 significantly attenuated release. In experiments below TP-induced changes to ATP release were sampled two minutes after the intervention.

Adenosine and ATP release. Fig 2 shows that adenosine attenuated (2 µM) or abolished (10 µM) TP-induced ATP release (left). Adenosine deaminase (AD) removed endogenous superfusate adenosine levels (not shown) and increased ATP release (right).

Discussion. ATP release from urothelium occurs when there is an increase of the transurothelial pressure (TP) gradient and also an increase of transurothelial potential. Adenosine (1-10 µM) attenuates ATP release induced by both modalities. Adenosine deaminase augmented TP-induced ATP release implying there are significant levels of endogenous adenosine. The block of ATP release by adenosine was reduced by A1-selective antagonists, but not A2-selective antagonists. Removal of endogenous adenosine also altered urothelial ion transport.

Conclusions. These results show that ATP itself has a positive feedback control over its own release, but through its breakdown products such as adenosine, exerts a negative feedback control. The overall effect will depend on the amplification exerted by the two pathways. The data also show an intimate connection between adenosine-receptor pathways, urothelial electrophysiology and ATP release. It remains to be shown if these pathways are altered in pathological conditions associated with increased ATP release.

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