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LUMINAL BUT NOT BASOLATERAL ATP AFFECTS UROTHELIAL TRANSEPITHELIAL POTENTIAL AND IONIC FLUX

Hypothesis / aims of study

ATP is released from the luminal (urinary) and basolateral (interstitial) surfaces of the urothelium in response to bladder filling and inflammation[1]. Basolateral ATP release may result in the direct stimulation of afferent nerves signaling the sensation of fullness or discomfort, but the role of luminal ATP release remains unknown. We hypothesized that luminal ATP release results in the activation of luminal P2 receptors which, when activated, increase the ionic flux across the urothelium. This electrical activity, starting within the luminal urothelium, could spread to the basolateral surface and lead to the release of neurotransmitters from the basolateral surface and downstream activation of the sensory nerves.

Study design, materials and methods

Freshly prepared guinea-pig bladders were isolated. The urothelium was dissected as a sheet from the underlying detrusor and mounted in an Ussing chamber. The urothelium was allowed to equilibrate under constant perfusion with a balanced salt solution (pH 7.26, and gassed with 95% O_2 and 5% CO_2) which was perfused over both surfaces. Differences in the Trans-epithelial Potential (TEP) in reference to the basolateral surface and in short circuit currents (SCC) were measured before and after the independent addition of exogenous purinergic and cholinergic agonists to the luminal and basolateral surfaces of the urothelium. Values are expressed as mean ± SEM of parameter differences.

Results

Baseline measurements (n = 56) yielded a TEP of –4.86 ± 0.32 mV and SCC of 2.08 ± 0.27 μA with a mean membrane conductance of 2.34 ± 0.30 milliSiemens (mS) (Fig 2). The addition of luminal ATP resulted in increased negativity of the TEP, and increased SCC, with a maximal effect at a concentration of 10^{-6} M, ΔTEP = -2.85 ± 0.15mV and Δ SCC = 0.4 ± 0.1μA (Fig 3). The addition of ATP to the basolateral urothelium had no discernable effect on TEP or SCC. The addition of carbachol to either the luminal or basolateral urothelium had no effect on TEP or SCC measurements.

Interpretation of results

The baseline negative transepithelial potential is indicative of an asymmetry of membrane transport proteins between the basolateral and luminal surface of the urothelium. ATP when released from the luminal surface of the urothelium may result in a P2X mediated flux of cations from the urine into the urothelium, which is manifest by increased SCC and increased TEP. However, this mechanism appears to be self-limiting because higher ATP concentrations reverse this effect. No ionic flux was noted when ATP was added to the basolateral surface or when cholinergic agonists were added to either surface. The former may reflect an asymmetry in the distribution of P2X receptors across the urothelium. The latter indicates a mechanism independent of acetylcholine/muscarinic receptors.

Concluding message

These data support the hypothesis that luminal release of ATP results in depolarisation of the urothelium starting from the luminal surface and spreading to the basolateral surface, potentially leading to increased neurotransmitter release from the basolateral surface. We believe that this constitutes a mechanism whereby a sensory signal, such as that generated by inflammation of the luminal urothelium, can be conveyed to the basolateral urothelium where sensory nerves are in proximity.

References

[1] Am J Physiol Renal Physiol 291: F332-F340, 2006.

Fig 1

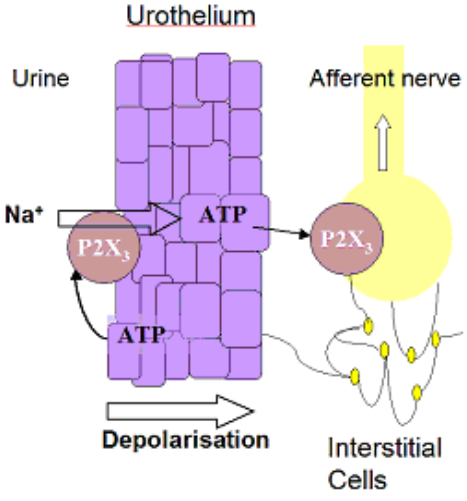


Fig 2

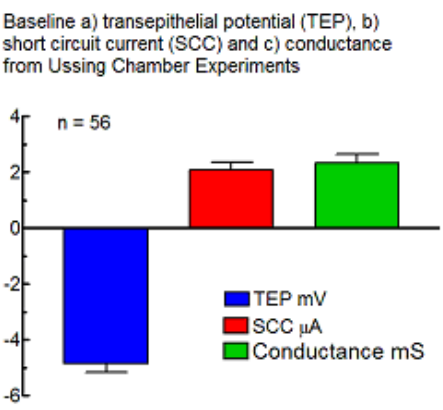
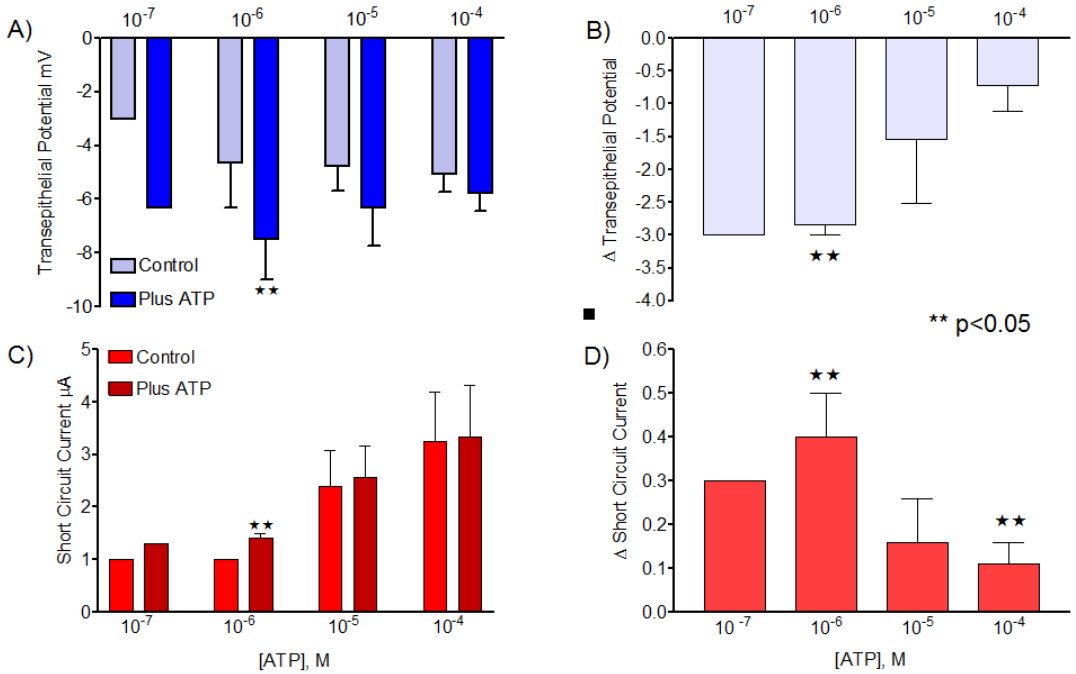


Fig 3

The effect of ATP when added to the apical urothelium a) Transepithelial Potential (TEP) b) Change in TEP c) Short Circuit Current (SCC) d) Change in SCC.



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Name of ethics committee	**Moorfields and Whittington Research Ethics Commitee**