ELECTROPHYSIOLOGICAL PROPERTIES AND INTRACELLULAR CA\(^{2+}\) REGULATION IN UROTHELIAL SUBJECTED TO STRETCH

Hypothesis / aims of study
Stretched bladder urothelium, as occurs when the bladder fills, results in the release of sensory transmitters such as ATP and acetylcholine [1,2]. Control of this release offers a pathway to moderate excessive sensations in some patients arising from the lower urinary tract during filling. However, the cellular pathways that control transmitter release are unknown, but it is known that the transmitters themselves, in particular ATP, generate a feedback control over further transmitter release. By analogy to other secretory cells changes to intracellular Ca\(^{2+}\) underlie transmitter release. We hypothesised that stretch of urothelial cells and exogenous ATP alter intracellular Ca\(^{2+}\) regulation in urothelial cells. The aim of the study was to test this hypothesis; to determine the intracellular pathways whereby interventions may alter Ca\(^{2+}\) regulation, and to characterise interacton between purinergic and cholinergic agonists.

Study design, materials and methods
Experiments were conducted in vitro using isolated basal urothelial cells and whole urothelial sheets from the guinea-pig bladder. Urothelial sheets were dissected free of the underlying detrusor muscle and either placed in Ussing chambers to measure electrophysiological properties, or disrupted by collagenase treatment into isolated cells. Small (<20 µm) cells were used to represent basal urothelial cells. Intracellular Ca\(^{2+}\) was measured by epifluorescence microscopy using the fluorochrome Fura-2, signals were calibrated for [Ca\(^{2+}\)]\(_i\) using a procedure described previously [3]. Membrane currents were measured under voltage clamp using a Cs-based filling solution to block outward currents. Urothelial transeurothelial potential (TEP) and short circuit current (SCC, current required to clamp TEP to 0 mV) were recorded via KCl-agar electrodes in the perfusion chambers opposite the two faces of the membrane. Data are expressed as median values [25%, 75% interquartiles]; differences between groups were tested using paired or unpaired non-parametric tests; the null hypothesis was rejected at p<0.05. Power calculations estimated n≥6 repeats was sufficient to detect a 40% change with 80% power.

Results
Cell stretch was generated by exposure to a superusisate of low osmolality ([Na] reduced from 147 to 88 mM), cells increased significantly in diameter to 113±7% of control, and control experiments were performed with a similar low-Na solution but with osmolality maintained by replacing removed NaCl with Tris-Cl. Low osmolality solution induced Ca\(^{2+}\) transients, with a ∆[Ca\(^{2+}\)]\(_i\) of 192 nM [115, 464]. The isosmolar low-Na solution generated a significantly (p<0.01) smaller ∆[Ca\(^{2+}\)]\(_i\) (39 nM [3, 85]). GdCl\(_3\) (100 µM, a blocker of stretch-activated ion channels) reversibly reduced the Ca\(^{2+}\) transient magnitude to 28% [12.9, 60.1] of control. By contrast, thapsigargin (500 nM, an agent that blocks Ca\(^{2+}\) uptake into intracellular Ca-stores) had no significant effect on the Ca\(^{2+}\) transient magnitude. Extracellular ATP (100 µM) also generated large Ca\(^{2+}\) transients (∆[Ca\(^{2+}\)]\(_i\)) 670 nM [317, 982]). In this case thapsigargin reduced the ATP-dependent Ca-transient to 17% [13, 29] of control. Pretreatment with the muscarinic agonist, carbachol (20 µM) had no effect on the above Ca\(^{2+}\) transients. Some isolated cells exhibited spontaneous oscillations of [Ca\(^{2+}\)]\(_i\) of magnitude comparable to those generated by low-osmolality and ATP interventions. Carbachol generated a small, but significant (p<0.05), reduction of frequency (0.52/min [0.40,0.69] vs 0.44/min [0.31, 0.52]), but had no effect on their amplitude. Simultaneous measurement of intracellular [Ca\(^{2+}\)]\(_i\) and membrane current revealed that the change of [Ca\(^{2+}\)]\(_i\) preceded the development of a large inward current during either exposure to ATP, UTP or UDP or the generation of a spontaneous event. ATP (100 µM) generated a significant increase of the urothelial TEP by -2.3 mV [1.8, 3.8] (apical vs basolateral surface) and SCC 0.30 µA [0.12, 0.50]. However carbachol, at concentrations between 1 µM and 3 mM, had no significant effect on either variable.

Interpretation of results
The data show that cells stretch, with hypoosmotic solutions, and ATP both generate transient increases of the intracellular [Ca\(^{2+}\)]\(_i\) in isolated basal urothelial cells. However, the cellular pathways where such a rise is effected are different. In the case of cell stretch the rise was Gd\(^{3+}\)-dependent and therefore probably represents Ca\(^{2+}\) influx from the extracellular space. By contrast, ATP-dependent transients were blocked by thapsigargin and this probably represent Ca\(^{2+}\) release from intracellular stores. We interpret the ATP data as activation via P2Y receptors from the electrophysiological data as: the effect of ATP was mimicked by the P2Y agonists UTP and; the rise of intracellular Ca\(^{2+}\) preceded an inward current, the latter thus an intracellular Ca\(^{2+}\)-activated current. By contrast, carbachol had very little effect on intracellular Ca\(^{2+}\) regulation and therefore it is postulated that any action is mediated by receptors that do not upregulate intracellular Ca\(^{2+}\) signalling. The effects of ATP and carbachol were mirrored in the Ussing chamber experiments using whole sheets of tissue. ATP increased the transepithelial potential and short circuit current, interpreted as augmenting transeurothelial ion transport, whilst carbachol had no effect.

Concluding message
Both cell stretch and exogenous ATP generate large intracellular Ca\(^{2+}\) transients in urothelial cells and offer a route whereby these interventions can modulate sensory transmitter release. However, the intracellular signalling pathways are different offering targets to selectively modulate the basal sensory pathway (urothelium stretch) and the positive feedback modulator (exogenous ATP). Carbachol had little significant actions suggesting that urothelial muscarinic pathways act through different cell signalling routes.

References

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