ALTERED ATP SIGNALLING IN UROTHELIUM OF OAB PATIENTS WITH EXACERBATED SYMPTOMS

Hypothesis / aims of study
Overactive bladder (OAB) is characterized by frequency, urgency and urge-incontinence, in the absence of urinary infection. A previously unrecognized pyuria (≥10 WBC/µl urine) in 35% of catheter urine specimens from patients diagnosed as having OAB has been reported to be associated with more severe symptoms (1). It is well established that extracellular ATP signalling, originating from stretch-evoked ATP release from the urothelium and necessarily involving activation of a variety of P2 receptors throughout the bladder, is involved in bladder sensation (2). Furthermore, inflammation is associated with increased ATP release from epithelial cells (3). Taken together, we hypothesise that in pyuric OAB patients, there is increased ATP release and/or increased P2 receptor expression, caused by inflammation, which ultimately results in increased sensory nerve excitation and the exacerbation of OAB symptoms.

Study design, materials and methods
Bladder urothelium biopsies were obtained from i) asymptomatic patients, ii) pyuric OAB patients, and iii) non-pyuric OAB patients, using flexible cystoscopy. Smooth muscle tissue was carefully removed from the biopsy sample using fine forceps and a dissection microscope. Basal and hypotonicity-evoked (i.e. stretch-evoked; to mimic bladder filling) ATP release from urothelium was quantified using a luciferin/luciferase assay, P2 receptor mRNA levels were investigated using quantitative real time-PCR, and P2 receptor expression was investigated in snap-frozen sliced tissue using immunohistochemistry. Data are mean±s.e.m.

Results
Basal ATP release was 50-fold greater from the urothelium of pyuric OAB patients (P<0.01; n=10) than from non-pyuric OAB (n=9) or asymptomatic patients (n=9). In contrast, in all three patient groups, the concentration of ATP released following stretch was similar. Basal and stretch-evoked ATP release was abolished from the urothelium of OAB patients with pyuria by the addition of the P2 receptor antagonist suramin (1 mM; n=5), whereas, only stretch-evoked ATP release was abolished by botulinum toxin-A (20 units/ml; n=2). In asymptomatic patient urothelium, we detected significant levels of P2X1,3,5,7 subunit and P2Y1,2,6,11,14 receptor mRNA (n=6). Urothelium from pyuric OAB patients showed a significant increase in abundance of P2X5 subunit and P2Y2,11,12 receptor mRNA, and a significant decrease in abundance of P2X7 subunit mRNA (P<0.01; n=3). Urothelium from non-pyuric OAB patients showed a significant increase in P2Y11 mRNA (P<0.01, n=3). Immunohistochemistry confirmed our real time-PCR data (n=3).

Interpretation of results
In summary, these data demonstrate that in a subset of OAB patients (those with pyuria) there is increased basal ATP release from the urothelium, which is abolished by the P2 receptor antagonist suramin, and, altered P2 receptor expression. These data suggest that increased ATP release from the urothelium, involving activation of P2 receptors, may play a role in the heightened symptoms associated with pyuric OAB patients.

Concluding message
The suppression of urothelial ATP signalling may represent a therapeutic target to treat the exacerbated symptoms experienced by OAB patients with significant pyuria.

References