135

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INCREASED ATP MEDIATED CONTRACTION OF ISOLATED DETRUSOR CELLS OF PATIENTS WITH OUTFLOW OBSTRUCTION

Hypothesis / aims of study

There has been considerable debate about the role of ATP signalling in the human detrusor. Early experiments contrasted with animal studies in finding minimal ATP expression in normal human detrusor. There some evidence that ATP may have a more prominent role in diseased states[1]. There is compelling evidence that inflammation of the urothelium should be associated with increased ATP release from urothelial cells[2]. In relation to other diseased states the data have relied heavily on full thickness bladder samples obtained from cystectomies and CLAM cystoplasties and thereby representative of highly select clinical groups. A new technique has been developed that extracts detrusor muscles cells in dispersions on microscope slides provides an opportunity to study detrusor pharmacology by using material obtained from flexible cystoscopic biopsies. This opens a portal into the wider populations that are suffering from more mundane lower urinary tract pathology. This studied compared the responses of dispersed detrusor myocytes to the purinergic agonist ATP and the muscarinic agonist carbachol. These experiments were conducted on tow groups of patients; one suffered from overactive bladder (OAB) and other outflow obstruction from benign prostatic hypertrophy (BPH)

Study design, materials and methods

Patients were recruited to the study with informed consent. OAB was diagnosed according to the ICS criteria and BPH was diagnosed with an International Prostate Symptom Score (IPSS) of >7 with an appropriate clinical presentation. The biopsies from the BPH group were collected at the time of a TURP or flexible cystoscopy. The samples from OAB patients were collected during routine cystoscopies. A bladder biopsy was incubated with an enzymatic dispersal solution containing papain and collagenase. The dispersed cells were transferred to slides, where varying concentrations of the muscarinic agonist carbachol or ATP were added before fixation. Digital photographs were taken of the dispersed cells, from which a sample of detrusor cell lengths were measured in reference to a microscopic graticule photographed and measured under the same conditions. For each slide a mean cell length was calculated from a sample of 50 to 100 cells, depending on the number extracted. Graphpad Prism 3 was used to fit a dose-response curve to the data by applying the Hill equation and the EC_{50} concentration was measured.

Results

Patients with outflow obstruction had a greater magnitude of contractile response with ATP than with carbachol (29 μm v 18 μm contraction) but at an identical potency (pEC_{50} = 6.8 for carbachol and for ATP Fig 1) Patients with OAB (Fig 2) showed a similar magnitude of contractile response with carbachol to those with outflow obstruction (19μm contraction) but at a lower potency (pEC_{50} =5.6) and showed no identifiable dose response with ATP.

Interpretation of results

In outflow obstruction there is a greater sensitivity to exogenous ATP and carbachol. It is speculative, but worth cautioning that the lack of an ATP response seen in OAB may not reflect an absence of receptor activity but have been due to high basal levels of endogenous ATP (possibly of urothelial origin), already being present in the cell suspension, and saturating out any further response. Studies using the purinergic antagonist Suramin would resolve this point. The pEC_{50} value for ATP obtained here is a thousand times lower than previously published values utilising muscle strips treated in organ baths. We believe that this is more representative of the working concentration of the neurotransmitter at the neuromuscular junction as in muscle strips there may be a significant diffusion gradient between the perfusing solution and the purinergic receptors located on the myocytes. Additionally, ATP may be rapidly broken down by ectopeptidases[3]. After all it would be rational to believe that ATP and acetylcholine are released in similar concentrations from the nerve terminals of the neuromuscular junction.

Concluding message

Myocytes from patients with bladder outflow obstruction showed an increased contractile response to ATP relative to patients with overactive bladder symptoms. The lack of ATP response in patients with OAB could reflect absence of active receptors or saturation of purinergic receptors with excessive endogenous ATP release. The experimental model is well up to supporting further studies which include specific antagonists as well.

Fig 1

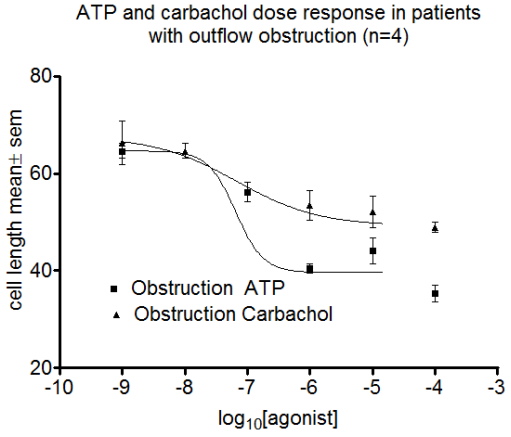
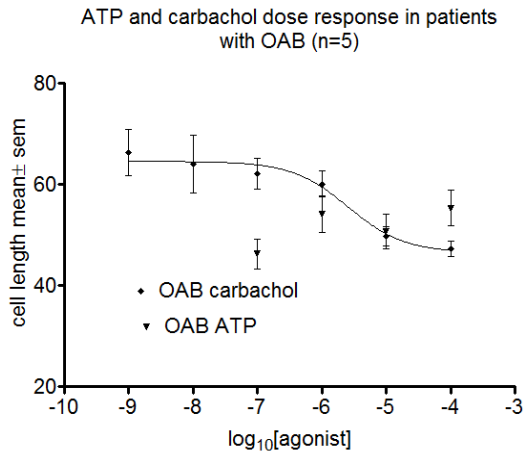


Fig 2



References

1. A quantitative study of atropine-resistant contractile responses in human detrusor smooth muscle, from stable, unstable and obstructed bladders, Bayliss et al 1999., 1999 J.Urol., vol. 162, no. 5, pp. 1833-1839
2. Augmented Strecth Activated Adenosine triphosphate release from bladder uroepithelial cells in patients with interstitial cystitis, Y. SUN, S. KEAY, P. De DEYNE, T. CHAI., The Journal of Urology, Volume 166, Issue 5, Pages 1951-1956
3. The contractile potency of adenosine triphosphate and ecto-adenosine triphosphatase activity in guinea pig detrusor and detrusor from patients with a stable, unstable or obstructed bladder Harvey, R. A. et al., 2002, J.Urol., vol. 168, no. 3, pp. 1235-1239

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Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**Whittington and Morrfields Research Ethics Committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**