332

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INTRAVESICAL ATP CONCENTRATION IN BLADDER ALIQUOTS AT 200 AND 400ML IN RELATION TO AFFERENT SENSATION DURING CYSTOMETRY AND BASELINE URINARY PH.

Hypothesis / aims of study

It is now well accepted that ATP released from the bladder urothelium in response to stretch [1] activates underlying afferent nerves and signals bladder fullness. Furthermore it is well known that low pH can stimulate ATP release *in vitro* [2]. In patients with Detrusor overactivity (DO) an inverse correlation between the intravesical concentration of ATP and the bladder volume at first desire to void (FDV) has previously been reported [3]. A similar correlation was not reported in control patients [3]. These correlations were seen in voided urodynamic fluid taken at the end of the cystometry test. The possibility that ATP was released during the voiding bladder contraction could not be evaluated.

The aim of our study was to 1) examine the concentration of ATP in intravesical fluid aliquots taken at bladder volumes of 200 and 400ml during filling cystometry and 2) to examine urinary pH in relation to ATP concentration in intravesical fluid.

Study design, materials and methods

Patients with a main complaint of either urge or stress leakage were recruited, those with mixed symptoms were excluded to facilitate study of the difference between women having marked urgency or no urgency. A midstream urine sample was taken for immediate urinary pH measurement. If any UTI symptoms were noted the patient was excluded. The patient was catheterised with a dual lumen catheter and saline was infused into the bladder at a filling rate of 75ml/ min. The volumes at first desire to void (FDV) and maximal cystometric capacity (MCC) were noted, as was the presence of any detrusor contractions during filling or provocation.

After removal of 10mL (deadspace), aliquots of intravesical fluid (5mL) were collected at bladder volumes of 200mL, 400 mL, as well as at MCC. The ATP concentration (nmoles/l) in these aliquots was determined immediately using a bioluminescence assay (Sigma). Data were expressed as median (interquartile range, IQR).

Patients were characterised as (a) DO (involuntary detrusor contractions during the filling phase which may be spontaneous or provoked) or (b) control (pure urodynamic stress incontinence, involuntary leakage of urine during increased abdominal pressure in the absence of detrusor contractions). Overall subject numbers comprised 23 DO and 10 control women. Recruitment into this study continues. As there is no pilot data a power calculation was unable to be performed.

Results: Cystometry with aliquot testing has been performed on 33 women (age 28-87 yrs).

A. Relationship between filling and intravesical ATP concentration

In both control and DO patients the ATP concentration at a bladder volume of 200mL was significantly higher than at 400mL (p=0.049, control; p=0.0058 DO, Wilcoxon Matched pairs test). The intravesical ATP concentration at 400mL and at MCC did not differ (P=1.0 Control, p=0.52 DO). Overall, intravesical ATP concentration did not differ between control and DO patients.

Table 1: ATP (nmol/L) in bladder aliquots in control and DO patients. (median, IQR)

	200mL	400mL	MCC
Control (N=10)	39.7 (24.5-66.7)	26.4 (16.8-59)	41.1 (14.8-51.1)
DO (N=23)	32.8 (12.5-96)	29.2 (12.8-52.1)	24 (8.8-48.2)

In the DO patients, the FDV ranged between 85 and 400mL. To check whether ATP levels differed in patients with very early First Desire, the DO patients were divided into three groups based on FDV less than 150mL [n=9], FDV = 150-250mL [n=8], FDV>250mL n=5). The concentration of ATP in intravesical fluid at 200mL was significantly higher in patients with a very early First Desire compared to those with a FDV greater than 250mL (Figure 1, p = 0.047). Analysis of the intravesical ATP concentration and bladder aliquot at 400mL did not show any relationship (p = 0.56).

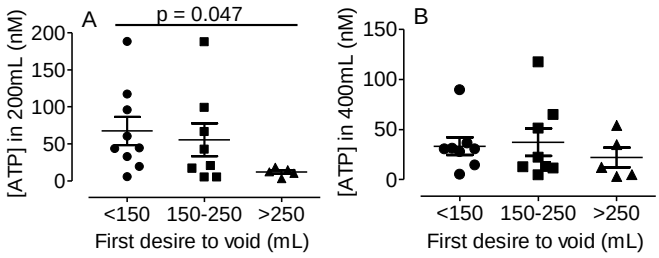

Figure 1. Relationship between volume at FDV and ATP concentration at 200mL (Panel A) and 400mL (Panel B)

B. Relationship between pH and intravesical ATP concentration

For all patients, at each of the bladder aliquots tested, there was a significant inverse correlation between urinary pH and the intravesical ATP concentration (fig 2A, 200mL, $r^2 = 0.24$, p = 0.009), 400mL (Fig 2B, $r^2 = 0.17$, p = 0.03) and at MCC (Fig 2C, $r^2 = 0.16$, p = 0.03).

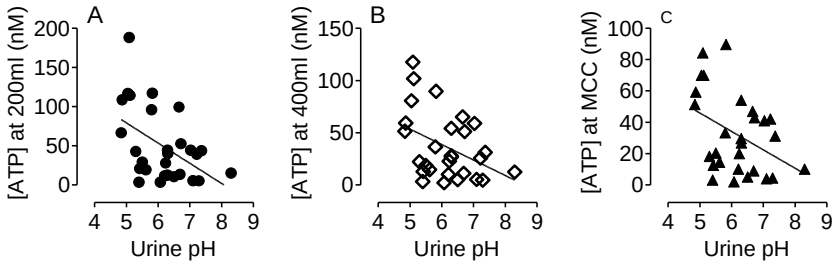

Figure 2. Relationship between urinary pH and ATP concentration at 200mL (Panel A), 400mL (Panel B) and at MCC (Panel C).

Interpretation of results

Surprisingly the concentration of ATP in intravesical fluid was highest at the lowest bladder aliquot measured (i.e. 200mL). This was unexpected, as ATP has been hypothesised to function as a trigger for bladder voiding (by activating afferent nerves after ATP release in response to bladder stretch). We expected to demonstrate an increase in the intravesical ATP concentration with increasing stretch of the bladder wall, i.e. that ATP would be highest at maximal capacity however this was not the case.

Interestingly in DO patients there was a significant relationship between the volume of FDV and the intravesical ATP concentration. We suspect that ATP plays a role in signalling the initial sensations of bladder filling, but not in the initiation of voiding. This would accord with previous reports of a correlation between ATP concentration in voided urodynamic fluid and FDV in DO patients [3].

More acidic urine pH was shown to significantly correlate with higher ATP release into intravesical fluid at all of the bladder aliquots examined. Increased acidity is known to stimulate ATP release [2]. Perhaps the acidic “internal milieu” bathing the urothelial cells prior to the start of the experiment has provoked greater sensitivity of these cells to stretch and thus enhanced the ATP release.

Concluding message

The relationship between bladder stretch during filling and ATP release appears to be more complex than was previously thought. This novel *in vivo* study further clarifies the important role of ATP in signalling the sensation of fullness, and the interplay with bladder pH.

References

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Specify source of funding or grant	**National Health and Medical Research Council**
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	**The St George Hospital Human Research Ethics Committee**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**