

## EFFECTS OF BLADDER OUTLET OBSTRUCTION ON ATP RELEASE FROM THE MUCOSA AND SPONTANEOUS CONTRACTIONS IN RAT BLADDERS

### Hypothesis / aims of study

Non-voiding contractions of bladders observed in a rat model of bladder outlet obstruction (BOO) imply that spontaneous contractile activity is increased by BOO. Recently there have been interests in the association between endogenous substances released from the mucosa and spontaneous contractions (SCs) although in-vitro SC is considered myogenic in nature. We have previously reported that a substance released from the mucosa activates SCs in bladder strips of rats, and the effect of this substance is more pronounced in bladder outlet obstruction (BOO). In that study, co-incubation of mucosa-denuded strips with mucosa strips reduced the potency of cromakalim, and in bladders with BOO this effect was positively correlated with bladder weight and the amplitude of SCs was increased by the co-incubation [1]. One of the candidates for the mucosa-derived substance that activates SCs is ATP. ATP is known to be released from the mucosa by stretch of bladder wall and enhance the autonomous contractile activity in isolated guinea-pig bladders [2]. However, the alteration in ATP release from the mucosa by BOO remains to be unknown. If ATP released from the mucosa is associated with SCs, it may be that there are some relationships between ATP release and SCs. The aim of the study was to examine the effects of BOO on ATP release from the mucosa and SCs, and correlate the ATP release with SCs in a rat model of BOO.

### Study design, materials and methods

Female Wistar rats (8 weeks old) were used in this study. The rats were divided into two groups; BOO group and sham-operation group. BOO was induced by incomplete urethral ligation (urethral outer diameter of 1.1 mm) (n=16). Sham-operation rats underwent only the dissection of urethra (n=20). Four weeks following the operation, bladders were removed and weighed. Two strips were created from each bladder body and weighed. In one of the two strips, the mucosa was removed (mucosa-denuded strip). The mucosa-denuded strips were also weighed and the % weight of the mucosa in mucosa-intact strips was calculated based on the difference in weight of the strip before and after removal of the mucosa.

The mucosa-intact and denuded strips were mounted in tissue baths, equilibrated at 1 g resting tension for at least 1 hour and washed with Krebs solution every 20 minutes. After SCs developed, the frequency and amplitude of SCs were recorded. The solution in the tissue baths were taken for the measurement of ATP just before the completion of the 20 minutes incubation. ATP was measured by the luciferin-luciferase assay. The estimated ATP release from the mucosa was calculated using the data on the ATP release from mucosa-intact and denuded strips, and the % weight of the mucosa for each bladder.

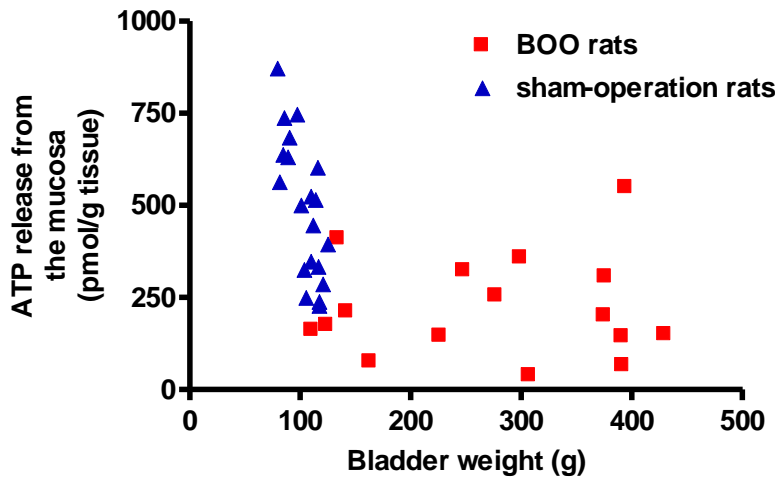
All values were expressed as the mean  $\pm$  standard error of the mean. Mann-Whitney U-test, Wilcoxon matched pairs test and linear regression test were used for statistical analyses, with a p-value of <0.05 considered statistically significant.

### Results

The amounts of ATP released from strips were  $221.1 \pm 18.7$  and  $38.5 \pm 8.3$  pmol/g tissue for the mucosa-intact and denuded strips, respectively, in the sham-operation group and  $83.3 \pm 9.8$  and  $22.7 \pm 6.4$  for those in the BOO group. The ATP release from the mucosa-intact strip was significantly greater in the sham-operation group than in the BOO group ( $p < 0.01$ ) while there was no statistical difference in the ATP release from mucosa-denuded strips between the two groups. The ATP release from mucosa-intact strips was significantly greater compared to that from denuded strips in the sham-operation group and the BOO group ( $p < 0.01$  for both). The % weight of the mucosa in mucosa-intact strips was approximately 40% ( $42.1 \pm 2.2\%$ ) in the sham-operation group and 30% ( $29.6 \pm 3.3\%$ ) in the BOO group. The estimated ATP release from the mucosa was significantly greater in the sham-operation group than in the BOO group ( $495.0 \pm 42.5$  vs.  $235.2 \pm 41.9$  pmol/g tissue,  $p < 0.01$ , figure).

The frequency of SCs was greater in the sham-operation group compared to the BOO group ( $6.30 \pm 0.16$  vs.  $4.30 \pm 0.35$  cycles/min. in mucosa-intact strips,  $p < 0.01$ ;  $6.36 \pm 0.15$  vs.  $4.90 \pm 0.57$  in denuded strips,  $p < 0.05$ ). The amplitude of SCs in mucosa-intact strips was higher in the sham-operation group than in the BOO group when values were corrected with weight of strips ( $53.7 \pm 3.5$  vs.  $28.8 \pm 5.1$  g/g tissue,  $p < 0.01$ ), and there was a trend that the amplitude in mucosa-denuded strips was decreased by BOO ( $28.3 \pm 3.2$  and  $23.0 \pm 5.3$  g/g tissue for the sham-operation group and the BOO group, respectively,  $p = 0.09$ ). In the BOO group, bladder weight was correlated negatively with the frequency of SCs in the mucosa-intact strip and the denuded strip ( $p < 0.01$  for both), but not with the amplitude irrespective of the presence or absence of the mucosa.

When correlating the estimated ATP release from the mucosa with bladder weight or SCs in the BOO group, ATP release from the mucosa was not correlated with bladder weight (figure), the frequency or the amplitude of SCs irrespective of the presence or absence of the mucosa.



**Fig. ATP release from the mucosa and bladder weight**

Interpretation of results

The estimated ATP release from the mucosa was reduced by BOO, and correlated with neither bladder weight nor the frequency and amplitude of SCs. This may indicate that the ATP release from the mucosa was impaired by BOO and not associated with SCs in-vitro. Therefore, ATP is not likely to be an endogenous substance released from the mucosa that activates SCs or reduces the effect of cromakalim on SCs in bladders with BOO as shown in a previous study [1]. BOO decreased the frequency of SCs irrespective of the presence or absence of the mucosa, and was likely to impair the contractile ability. These imply the detrusor alterations induced by BOO.

Concluding message

Stretch-induced ATP release from the mucosa in rat bladders is decreased by BOO and may not be associated with SCs in this model of BOO. The decreased frequency and amplitude seem to be remarkable features of SCs in-vitro in bladders with BOO.

References

1. Akino H et al. abstract #438, ICS annual meeting 2009.
2. Gillespie JI et al. BJU Int 93; 393, 2004.

<b>Specify source of funding or grant</b>	None
<b>Is this a clinical trial?</b>	No
<b>What were the subjects in the study?</b>	ANIMAL
<b>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</b>	Yes
<b>Name of ethics committee</b>	Animal Care and Use Committee of the University of Fukui