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ATP AND PGE2 RELEASE FROM THE RAT BLADDER IN-VITRO AND IN-VIVO: THE EFFECT OF BLADDER OUTLET OBSTRUCTION AND THE ASSOCIATION WITH BLADDER WEIGHT, SPONTANEOUS CONTRACTIONS OR NON-VOIDING CONTRACTIONS

Hypothesis / aims of study

We have previously reported that a diffusible substance released from the mucosa may enhance spontaneous contractions (SCs) and the effect of the substance is pronounced by bladder outlet obstruction (BOO) [1]. ATP and PGE_2 are likely to be the candidates of the substance, further ATP and PGE_2 are considered to be involved in the generation of normal bladder sensation and also urgency or bladder pain. However, little is known about the effect of bladder outlet obstruction (BOO) on ATP or PGE_2 release from the bladder in an animal model of BOO. The aim of the study was to examine the amount of ATP and PGE_2 released not only from the bladder strip in-vitro but also from the inner surface of the bladder in-vivo in rats with and without BOO, and correlate them with bladder weight, SCs in-vitro or non-voiding contractions (NVCs) in-vivo.

Study design, materials and methods

Female Wistar rats were used in the study. BOO was induced by incomplete urethral ligation. Sham-operated rats underwent only the dissection of the urethra. In-vitro experiment: Four weeks following the operation, bladders were removed and weighed. The mucosa-intact and denuded strips from each bladder body were isolated and weighed, and 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. ATP and PGE_2 release from the bladder strip during the 20 minutes incubation were measured with the luciferin-luciferase assay and the enzyme immunoassay, respectively. In-vivo experiment: Four weeks following the operation a cystostometrogram (CMG) catheter was implanted. One week after the CMG catheter implantation bilateral ureters were cut to prevent urine from entering the bladder. After the maximum bladder capacity was determined with repeated CMG, the bladder was distended with saline to 80% maximum capacity and then the intravesically infused saline was collected for the ATP and PGE_2 measurement. Finally bladders were removed and weighed. The amount of ATP and PGE_2 were normalized to bladder weight in both in-vitro and in-vivo experiment. The data were expressed as mean ± SEM.

Results

In-vitro experiment: Both ATP and PGE_2 release were significantly reduced in rats with BOO (BOO rats) compared to sham-operated rats except for the ATP release from the mucosa-denuded strip (Table 1). ATP was mainly released from the mucosa while PGE_2 was mainly released from the detrusor (Table 1). The frequency and amplitude of SCs were significantly decreased in BOO rats compared to sham-operated rats ($p<0.05$ for both). PGE_2 release was inversely correlated with bladder weight irrespective of the presence or absence of the mucosa in BOO rats ($p<0.01$). Correlating ATP and PGE_2 release with SCs, it was found that PGE_2 was positively correlated with the frequency and amplitude of SCs in both of mucosa-intact and denuded strips in BOO rats ($p<0.05$), but this was not the case with ATP.

Table1. ATP and PGE_2 release in-vitro in BOO rats and sham-operated rats.

	mucosa-intact strip		mucosa-denuded strip	
	BOO (n=9)	Sham (n=8)	BOO (n=9)	Sham (n=8)
ATP (pmol/g tissue)	102 ± 14*	268 ± 27	31 ± 12	27 ± 14
PGE_2 (ng/g tissue)	46 ± 13*	139 ± 17	46 ± 9*	131 ± 11

*$p<0.01$ compared to sham-operated rats

In-vivo experiment: Contrary to the results of the in-vitro experiment, BOO increased the amount of ATP and PGE_2 released from the inner surface of the bladder (Table 2) and PGE_2 release was likely to be increased as the bladder weight was increased in BOO rats (Figure 1). Non-voiding contractions (NVCs) were observed on CMG in all BOO rats, but not in sham-operated rats. Significant association of ATP or PGE_2 release with NVCs was not observed in BOO rats.

Table2. ATP and PGE_2 release in-vivo[†] in BOO rats and sham-operated rats.

	BOO (n=10)	Sham (n=7)
ATP (pmol/g tissue)	128 ± 40*	30 ± 8
PGE_2 (pg/g tissue)	191 ± 76*	38 ± 10

[†] The amount of ATP and PGE_2 were measured when the bladder was distended to 80% maximum bladder capacity. *$p<0.05$ compared to sham-operated rats

Interpretation of results

The decreased level of ATP and PGE_2 release in-vitro and the inverse correlation between PGE_2 release in-vitro and bladder weight implies an impairment of the bladder wall caused by BOO. PGE_2 release in-vitro was associated with SCs but not ATP release, suggesting that PGE_2, rather than ATP in the mucosa, is more associated with the generation of SCs in the obstructed bladder.

It is difficult to explain why ATP and PGE_2 release in-vivo were increased in BOO rats despite of the decrease in ATP and PGE_2 release in-vitro. A major difference between the in-vivo and in-vitro experiments is the intact intramural neural network in the bladder with connections to the central nervous system. Therefore, the difference in the results of ATP and PGE_2 release between the in-vivo and in-vitro experiments suggested a possible contribution of the intact neural network to the ATP and PGE_2 release from the bladder. As it is considered that intravesically released ATP and PGE_2 may be derived from the mucosa, the generation of ATP and PGE_2 in the mucosa in-vivo may be increased in the obstructed bladder although PGE_2 seemed to be mainly generated in the detrusor muscle.

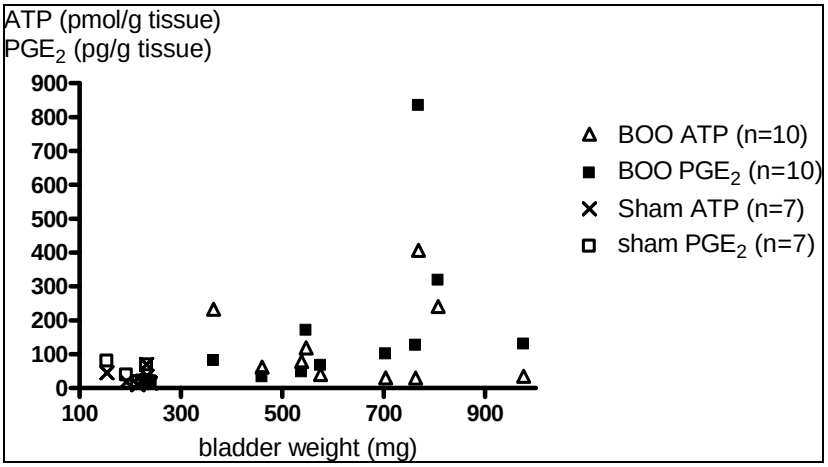

Figure 1 Scatter plot of ATP and PGE_2 release from bladders in-vivo as a function of bladder weight in BOO rats and sham-operated rats

Concluding message

ATP and PGE_2 release from the bladder strip in-vitro were reduced by BOO. This implies an impairment of the bladder wall due to BOO. PGE_2, rather than ATP in the mucosa, may be more associated with the generation of SCs in-vitro in this model of

References

1. Akino H, et al. AUA 2008 abstract # 355

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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