ATP release from bladder urothelium and serosa in a rat model of partial bladder outlet obstruction

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ABSTRACT

Overactive bladder is one of the major health problems especially in elderly people. Adenosine triphosphate (ATP) is released from urinary bladder cells and acts as a smooth muscle contraction and sensory signal in micturition but little is known about the role of ATP release in the pathophysiology of overactive bladder. To assess the relationship between ATP and overactive bladder, we used a partial bladder outlet obstruction (pBOO) model in rats. The bladder caused several changes by pBOO: An increase in bladder weight, hypertrophy of sub-urothelium and sub-serosal area, and frequent non-voiding bladder contraction during urine storage. Basal ATP release from urothelium and serosa of pBOO rats was significantly higher than that of normal rats. Distention-induced ATP release from urothelium of normal and pBOO rats had no significant change. However, distention-induced ATP release from serosa of pBOO rats was higher than that of normal. These findings may identify ATP especially released from serosa as one of causes of non-voiding contractions and overactive bladder symptoms.

Overactive bladder is a major health problem and nocturia, one of the common symptoms in elderly people, leads to sleeplessness, daytime sleepiness, depression, and cognitive dysfunction, which reduce the quality of daily life (7, 12). The characteristic issue in the urodynamics of the bladder over activity has been known to be involuntary non-voiding bladder contraction, which causes urinary urgency. However, the mechanisms underlying bladder over activity have been poorly understood. In many preclinical studies, a partial bladder outlet obstruction (pBOO) model in rats has been used to study bladder over activity (19, 20). Partial obstruction of the lower urinary tract results in morphological and pathological changes in the bladder, such as hypertrophy, compensation, and decompensation, as well as urodynamic change including non-voiding contraction (19, 20).

ATP is one of the most important neurotransmitters for normal function of the urinary bladder in the physiological condition and has been known to induce non-voiding bladder contraction in pathological conditions (14, 27). ATP is released from the pelvic postganglionic nerve ending with acetylcholine and activates the P2Y receptors in the bladder smooth muscle to lead the initial bladder contraction in the process of micturition (3, 6, 21). ATP is also released from urothelial cells during bladder stretching (2, 8, 29) or upon activation of the transient receptor potential (TRP) V1 channel, muscarinic acetylcholine receptors, or 5HT receptors, and activates P2X receptors on the afferent nerve terminals (2, 23). These P2X receptors can elicit the activity of the afferent nerves during the storage of urine and most likely trigger the micturition reflex (28). In pathological conditions, including spinal cord injury and chemotherapy-induced cystitis, ATP is re-
leased from fibroblast cells and/or mast cells (9), and induces non-voiding contraction in the bladder (14, 27). In pBOO model, hypertrophy occurs in sub-urothelial area and sub-serosal area with accumulation of inflammatory cells (mast cells) and fibroblasts (25), both of which can release ATP.

The current study examined, first, our pBOO model condition physiologically and histologically; second, whether pBOO affects basal and pressure-evoked ATP release in the urothelium or/and serosa of the bladder, by measuring ATP release from the isolated bladder tissues.

MATERIALS AND METHODS

Animals. Six to 8-weeks-old female Sprague-Dawley rats were used in this study (n = 55, 150–200 g at arriving). All the protocols were approved by the Animal Research Committee of Akita University (Akita, Japan), and followed the American Physiological Society guidelines for animal research.

Partial bladder outlet obstruction rat model. Under 2% halothane anesthesia, a lower midline incision was made in the rats. The prevesical fat tissues were retracted to expose the bladder neck and urethra. The urethra was ligated to a 1.1 mm stainless pipe using a 4-0 silk suture. The pipe was then removed, and the incision was closed with layers to layers. Two weeks after the surgery, the rats were euthanized by cervical dislocation with 5% halothane, and the bladder was isolated; 200–500 mg bladders were used for the study (n = 24). Naive rats were also used as normals (n = 31).

Bladder cystometry (CMG) in the pBOO rats. Two weeks after the pBOO surgery, a lower midline incision was made under 2% sevoflurane anesthesia to expose the urinary bladder. A PE-50 polyethylene catheter was inserted into the apex of the bladder for bladder filling and pressure recording. The catheter was secured in place using a 5-0 nylon suture. The catheter was routed subcutaneously to the back of the neck, and the bladder neck ligature for outlet obstruction was removed. Next day, the bladder catheter was opened. The animal was placed in a Ballman cage (Natsume Seisakusho Co. Ltd., Tokyo, Japan), and the catheter was connected to one part of a pressure transducer. The other part of the transducer was connected to a syringe pump. Isotonic saline (0.9% NaCl) was continuously infused into the bladder at a rate of 230 μL/min. Bladder pressure was recorded on a AP601 polygraph (Nihon Koden, Tokyo, Japan) and digitized using a converter for recording on PowerLab 4/30 (ADInstruments Pty. Ltd., NSW, Australia).

Histological study of control and pBOO bladder. Paraffin blocks were sectioned (2 μm). The slices were mounted on slides and stained with hematoxylin and eosin using standard procedures. Photos were taken using a digital camera (Canon, Tokyo) mounted on a microscope (Olympus, Tokyo) using × 100 optical magnification.

ATP release assay using the Ussing chamber. ATP release assay was performed as described previously (22, 23). In brief, the isolated urinary bladders were opened vertically from the urethra to the apex. The opened bladder was then mounted to act as a 9.4 mm$^2$ or 15.7 mm$^2$ (serosal ATP release assay) area diaphragm between the two halves of the chamber. The chambers were then filled with oxygenated (95% O$_2$/5% CO$_2$) Krebs solution (117 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl$_2$, 2.5 mM CaCl$_2$, 24.8 mM NaHCO$_3$, 1.2 mM NaH$_2$PO$_4$, and 11.1 mM glucose). To assess the distention-evoked ATP release, we then applied hydrostatic pressure (0, 10 or 30 cmH$_2$O) to the one half of the chamber (opposite side of the measurement side) for 40 min. Krebs solution (50 μL) was introduced into the measurement side of the chamber before and after pressure application, and the ATP content was measured using the luciferin-luciferase method (Kikkoman Co. Ltd., Tokyo, Japan) according to the manufacturer’s protocol. The amount of ATP release was determined using standard curves constructed for each experiment using 3 × 10$^{-7}$, 3 × 10$^{-8}$, 3 × 10$^{-9}$, and 3 × 10$^{-10}$ M ATP.

Data analysis. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria) (16). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics. Data were analyzed using the Student’s t test, Mann-Whitney U test or one-way ANOVA with post-hoc Holm’s multiple comparison test. Statistical significance was assigned to differences with P values less than 0.05. All the data are expressed as mean ± standard error of the mean (S.E.M.).
ATP release from bladder

Urothelial ATP release induced by physical distention

We then examined distention-evoked urothelial ATP release from the baseline, using 10 or 30 cmH₂O physical distention for 40 min as a stimulus (Fig. 3). Without distention (0 cmH₂O), only small changes in urothelial ATP release were observed in normal and pBOO rats (0.19 ± 0.11 nM, n = 8 and −0.4 ± 0.12 nM, n = 5, respectively) and there was no significant difference between those values (P = 0.64). Upon 10 cmH₂O distention, changes in the urothelial ATP release from the baseline had no significant increase in both normal (0.31 ± 0.07 nM, n = 8, P = 0.99) and pBOO rats (1.13 ± 0.87 nM, n = 5, P = 0.44) compared to non-distention groups, and there was no significant difference between these distention-evoked ATP release (P = 0.58).

Bladder weight, cystometry and histology

The bladder weight in rats 2 weeks after pBOO surgery (289.3 ± 18.3 mg, n = 29) was significantly larger than that in normal rats (71.5 ± 3.51 mg, n = 24, P < 0.001) (Fig. 1A).

Bladder cystometry and histological analysis were performed to confirm the effects of pBOO on bladder histology and the urodynamic (Fig. 1B and C). Consistent with previous reports (19, 20, 25), frequent non-voiding bladder contraction during urine storage and hypertrophy in sub-urothelial and sub-serosal areas were observed in rats 2 weeks after pBOO.

Basal ATP release from urothelium and serosa

Basal ATP release from the urothelium in pBOO rats before applying the pressure (6.72 ± 0.19 nM ATP, n = 5) was significantly higher than that of normal rats (3.97 ± 0.26 nM, n = 8, P < 0.001) (Fig. 2). Similarly to the result in the urothelium, basal ATP release from the serosa in pBOO rats (5.71 ± 1.03 nM, n = 4) was significantly higher than that of normal rats (1.60 ± 0.61 nM, n = 4, P = 0.01) (Fig. 2).

RESULTS

Bladder weight, cystometry and histology

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Bladder cystometry and histological analysis were performed to confirm the effects of pBOO on bladder histology and the urodynamic (Fig. 1B and C). Consistent with previous reports (19, 20, 25), frequent non-voiding bladder contraction during urine storage and hypertrophy in sub-urothelial and sub-serosal areas were observed in rats 2 weeks after pBOO.

Fig. 1 Change in bladder by partial bladder outlet obstruction (pBOO). A: Bladder weight in normal and 2 weeks after pBOO. P < 0.001 vs. control; Mann-Whitney U test. B: Representative microphotographs of hematoxylin eosin staining in normal and pBOO bladder. Mucosal surface is top. C: Cystometrogram (CMG) of pBOO rat. A representative result of CMG. Non-voiding pressure elevation was frequently observed. Micturition pressure is 14.2 ± 0.47 cmH₂O, maximum pressure is 40.5 ± 0.75 cmH₂O.

Urothelial ATP release induced by physical distention

We then examined distention-evoked urothelial ATP release from the baseline, using 10 or 30 cmH₂O physical distention for 40 min as a stimulus (Fig. 3). Without distention (0 cmH₂O), only small changes in urothelial ATP release were observed in normal and pBOO rats (0.19 ± 0.11 nM, n = 8 and −0.4 ± 0.12 nM, n = 5, respectively) and there was no significant difference between those values (P = 0.64). Upon 10 cmH₂O distention, changes in the urothelial ATP release from the baseline had no significant increase in both normal (0.31 ± 0.07 nM, n = 8, P = 0.99) and pBOO rats (1.13 ± 0.87 nM, n = 5, P = 0.44) compared to non-distention groups, and there was no significant difference between these distention-evoked ATP release (P = 0.58).
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ATP release, even without distention, from not only the urothelium but also the serosa (Fig. 2). Urothelial ATP release upon physical distention was increased according to the distention intensity in both the control and the pBOO rats. However, no significant difference of the increase in urothelial ATP release was observed between the control and pBOO rats upon physical distention, but tended to increase in 10 cmH₂O and 30 cmH₂O (Fig. 3). On the other hand, serosal ATP release upon physical distention in pBOO rats was higher than normal rats (Fig. 4). The increase in ATP release from the urothelium without distention suggests that pBOO causes changes on the urothelial side of the bladder. Consistent with this result, a previous clinical study showed that the urinary ATP in patients with overactive bladder syndrome was increased (5, 18). This result also suggests that our findings are true for humans as well.

In addition, the increase in ATP release from the serosa means that pBOO affects the serosal side of bladder. Comparing the rate of basal ATP release by pBOO between urothelium and serosa, pBOO may strongly affect to serosal side of the bladder rather than urothelial side. Histologically, pBOO causes hypertrophy of the connective tissue and smooth muscles due to bladder inflammation and fibrosis (25). Given that ATP can be released from mast cells and fibroblasts, our findings indicate that sub-urothelial or sub-serosal cells may play a significant role in the release of ATP from the urinary bladder in pBOO rats (9). Many studies were accomplished in the past about the functional change of bladder urothelium caused by pBOO, but there were few studies about the functional change of bladder serosa by pBOO. The current study revealed that the bladder function-
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al change caused by pBOO released ATP from serosal side of the bladder.

The release of ATP from the urothelium upon physical distention is a well-known occurrence (22, 23). In our study, the change in the ATP increment caused by physical distention (10 and 30 cmH2O) was not significant between the control and pBOO rat uroethelium. In contrast, serosal ATP release evoked by physical distention (10 cmH2O) was significantly increased by pBOO. A previous study revealed that number and mass of nerve fibers are reduced in pBOO model and patients (10, 13). This denervation is estimated to be a one of causes of overactive bladder. ATP release from sub-serosal area is an important factor for smooth muscle contraction during urine storage and activation of afferent nerve terminals. While urine storage, ATP released from serosal side of expanded bladder also may play an important role in pathophysiology of overactive bladder.

Little is known about the mechanisms about bladder activity. Several ion channels such as TRPM7 (30) or PIEZO1 (26) have also been known to be involved in bladder activity. However, pBOO did result in an increase in bladder weight (Fig. 1A) and in the hypertrophy of the sub-urothelial and sub-serosal area (Fig. 1B). Moreover, bladder cystometryography revealed frequent non-voiding bladder pressure elevations during urine storage (Fig. 1C). It was reported that blockade of muscarinic or β3 adrenergic receptors and inhibition of type 4 phosphodiesterase decrease the number and amplitude of non-voiding contractions in pBOO rats (11, 15). In our study, this bladder overactivity might be caused by ATP released from urothelium and bladder serosa. In a clinical study of overactive bladder patients, bladder with low first desire to void releases more ATP than the bladder with higher first desire to void (4). This result suggests that our hypothesis may be true for humans as well. Under physiological conditions, the ATP released from the urothelium activates P2Y receptors in the smooth muscle area of the bladder, which triggers the initial contraction in the process of micturition (3, 6, 21). However, in pBOO, bladder contraction might be triggered by sub-serosal ATP release. We hypothesized that this ATP may have been released mainly from the sub-serosal inflammatory cells and fibroblasts.

In summary, our findings indicate that pBOO affects the sub-serosal or sub-urothelial cells of the urinary bladder and increases ATP release from these cells. This ATP release may trigger non-voiding bladder pressure elevation.

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