306

Matsumoto-Miyai K[1], Kagase A[1], Murakawa Y[1], Yoshizumi M[1], Kawatani M[1]
1. *Department of Neurophysiology, Akita Univeresity School of Medicine*

THE STORE-OPERATED CA2+ ENTRY NEGATIVELY REGULATES THE DISTENTION-ELICITED ATP RELEASE FROM UROTHELIUM

Hypothesis / aims of study

The epithelium in urinary bladder (urothelium) serves as a sensory organ in micturition/nociception pathway by releasing ATP in response to mechanical/chemical stimuli. The excessive ATP release could result in the symptoms of overactive bladder. Previous studies showed that an increase in cytosolic Ca^{2+} from the extracellular space and the endoplasmic reticulum (ER) could regulate the urothelial ATP release. However, its regulatory mechanism remains controversial (1, 2). Here we investigated the effect of the Ca^{2+} influx or the Ca^{2+} release from the ER on the distention-induced ATP release from urothelium using the Ussing chamber assay.

Study design, materials and methods

The urinary bladders were dissected from 8-12 week-old C57BL/6 or B6;129 mice. Opened bladder wall was mounted to act as a diaphragm of 7 mm^2 between two halves of a customized small Ussing chamber. Chambers were filled with Krebs solution bubbled by 95% O_2/5% CO_2. We applied hydrostatic pressures from the smooth muscle (serosal) side for 20 min in order to induce the distention of bladder wall. 50 µl of Krebs was sampled from the urinary (mucosal) side. The ATP content was assayed by means of luciferin-luciferase method (Kikkoman Co. Ltd., Japan) according to the manufacturer's protocol. Standard lines were constructed using 3×10^{-7}, 3×10^{-8}, 3×10^{-9}, and 3×10^{-10} M of ATP in each experiment. Agonists/antagonists were administrated 20 or 30 min before the application of pressure. In another experiment, we administrated 2-aminoethyl-diphenylborinate (2-APB) between the 1st and 2nd pressures, and evaluated the ratio of 2nd ATP release to 1st one from the same bladder wall. All data were expressed as the mean ± SEM.

Results

The ATP release from the mucosal side was increased in proportion to the degree of hydrostatic pressure (15-45 cm H_2O; Figure 1A), which was not inhibited by tetrodotoxin (1 µM). The inhibition of Ca^{2+} release from the ER by inositol 1,4,5-triphosphate (IP_3) receptor antagonist, heparin (100 µg/ml), abolished the distention-induced ATP release (Figure 1B). In consistent, ryanodine receptor agonist, caffeine (10 mM), elicited the small but significant ATP release without the pressure. Interestingly, the removal of Ca^{2+} from Krebs enhanced the ATP release (Figure 2A), although the degradation velocity of exogenous ATP at mucosal side was unchanged (Figure 2B). The blockade of store-operated Ca^{2+} channels by 2-APB (100 µM) also enhanced the distention-elicited ATP release from the same urothelium, only when the extracellular Ca^{2+} was present (Figure 3).

Interpretation of results

Our previous study indicated that the differentiated urothelial cells have the less potential to release ATP. Taken together, the present results indicate the distention of bladder wall elicited the urothelial ATP release from basal and intermediate cells, not from mantle cells. The effects of heparin and caffeine showed that the Ca^{2+} release from the ER was essential for the induction of ATP release. On the other hand, the presence of extracellular Ca^{2+} reduced the ATP release itself, because the removal of extracellular Ca^{2+} did not affect the ecto-ATPase activity. The effect of 2-APB indicated that this reduction resulted from the store-operated Ca^{2+} entry from the extracellular space, which follows the depletion of Ca^{2+} store in the ER.

Concluding message

The Ca^{2+} bidirectionally regulates the urothelial ATP release. Whereas the Ca^{2+} release from the ER triggered the ATP release from urothelium, the store-operated Ca^{2+} entry from the extracellular space reduced the level of ATP release, and might adjusted it to the physiologically adequate level. These data imply that the regulation of store-operated Ca^{2+} channels such as TRPC family is a potential therapeutic target for the overactive bladder.

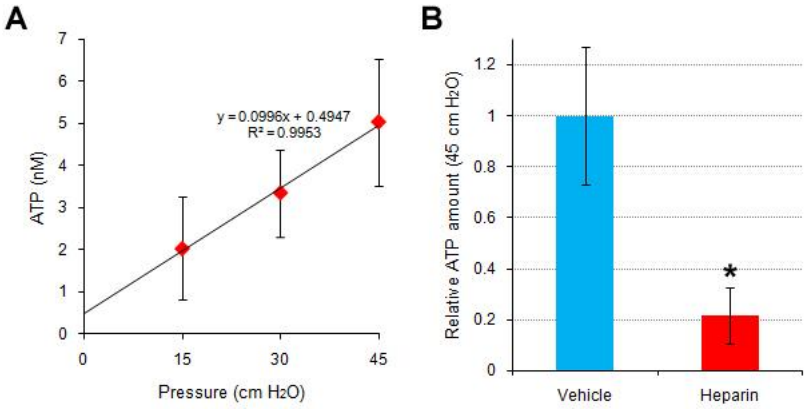

Figure 1. The Ca^{2+} release from the ER was essential for the urothelial ATP release.
A: The distention of bladder wall elicited the urothelial ATP release in proportion to the hydrostatic pressure.
B: Blockade of IP_3 receptor by heparin inhibited the distention-elicited ATP release. The average of ATP amount in vehicle was set to 1. *; $p < 0.05$ vs vehicle by unpaired t test.

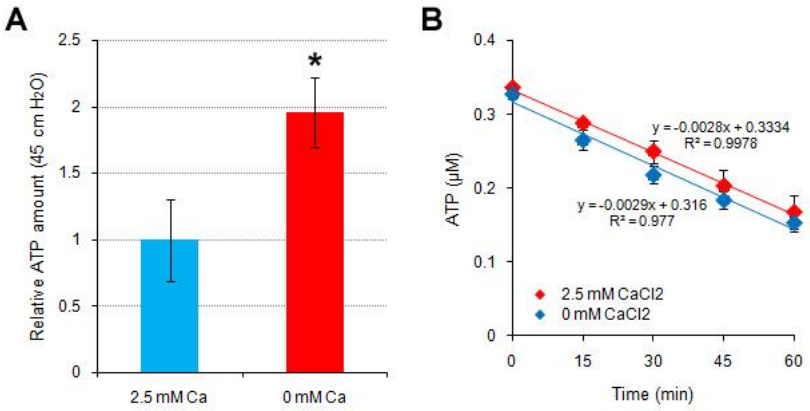

Figure 2. The extracellular Ca^{2+} reduced the level of urothelial ATP release.
A: Removal of extracellular Ca^{2+} enhanced the distention-induced ATP release up to 2-fold. The average of ATP at 2.5 mM Ca^{2+} was set to 1. *; $p < 0.05$ vs 0 mM by unpaired t test.
B: Removal of extracellular Ca^{2+} did not affect the degradation of exogenously-applied ATP (0.3 μM) at mucosal side without the pressure.

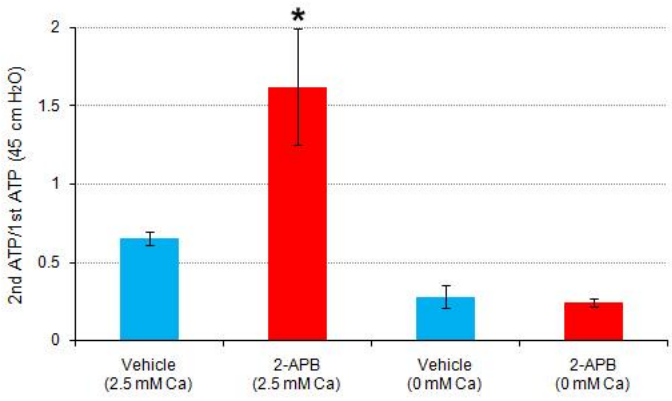

Figure 3. The store-operated Ca^{2+} entry negatively regulated the urothelial ATP release.
The store-operated Ca^{2+} channel blocker, 2-APB, enhanced the 2nd distention-induced ATP release only when the extracellular Ca^{2+} was present. *; $p < 0.05$ vs vehicle by unpaired t test.

References

1. Ferguson, D.R., Kennedy, I., Burton, T.J. 1997. ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes--a possible sensory mechanism? J Physiol 505 (Pt 2), 503-511
2. Birder, L.A., Barrick, S.R., Roppolo, J.R., Kanai, A.J., de Groat, W.C., Kiss, S., Buffington, C.A. 2003. Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium. Am J Physiol Renal Physiol 285, F423-429

Specify source of funding or grant	**None**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed	**Yes**

or ethical committee approval obtained?	
Name of ethics committee	**Animal Care Committee in Bioresearch-Education Centre, Akita University**