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AMILORIDE-SENSITIVE ION CHANNELS MODULATE THE STRETCH-EVOKED ATP RELEASE FROM THE RAT BLADDER EPITHELIUM

Hypothesis / aims of study

The increase of afferent activity is one of the possible mechanisms for overactive bladder symptoms (OAB). However, the precise mechanisms by which mechanical stimuli excite bladder afferents remain unclear. In response to stretch, the urinary bladder epithelium releases some neurotransmitters, including ATP. The released ATP will activate the $P2X_3$ receptors on the sensory nerve terminals running beneath and within the bladder epithelium, finally resulting in the activation of bladder afferents. Some mechanosensory molecules in the epithelial cells are supposed to sense the stretch applied to the urothelium, and then modulate ATP release.

Epithelial Na^+ channel (ENaC) is a candidate for mechanotransducer in various species. ENaC is up-regulated in the bladder epithelium of patients with infravesical obstruction and OAB (1). As we presented at the last annual meeting, intravesical infusion of antagonist of ENaC, amiloride, increased the bladder capacity of rats. In the present study, we examined the effect of amiloride on the stretch-evoked ATP release from the rat urinary bladder strips.

Study design, materials and methods

The urinary bladders were harvested from the 11 w SD female rats. The bladder strips with or without epithelium were incubated in Magnus tube filled with 2.5 ml Krebs solution (37 ºC, 95 % O_2 and 5 % CO_2 bubbled), and pre-suspended with the use of 1gram tension. After 1 hour of equilibration, the strips were subjected to stretch with an increase of either 30 % or 50 % of their original lengths. Every 25 µl Krebs solution was sampled before and after stretch. The ATP amount was measured with the use of luciferin-luciferase assay, and calculated from the standard curve established each time (R^2=0.999-1.000). The effect of amiloride on the stretch-evoked ATP release was investigated by adding 1 mM amiloride into the Krebs solution. The ATP amount released after stretch was expressed as the rate (%) of increase over basal release.

Results

The ATP amounts released from whole layer strips with the epithelium (basal release, 30 % stretch, 50 % stretch = 2634, 3450, 12303 fmol/g) were significantly higher those from strips without epithelium (210, 220, 336 fmol/g). In the whole layer strips, the difference in ATP release between basal and 50 % stretch was significant (n = 8, p = 0.03). The dominant source (over 90 %) of stretch-evoked ATP release was the mucosa, thus only whole layer strips were used in the following experiments.

1 mM amiloride didn't affect the basal ATP release from the whole layer strip (control: amiloride = 2634:3807 fmol/g, n= 15, p = 0.18). Amiloride decreased the % increase of ATP release by 30 % stretch without significance, from 131 % in control to 6 % in 1 mM amiloride (n = 7, p = 0.13) (Fig 1). When stretched by 50 % lengths, amiloride significantly attenuated % increase of ATP release from 443 % to 22 % (n = 7, p =0.01) (Fig 1).

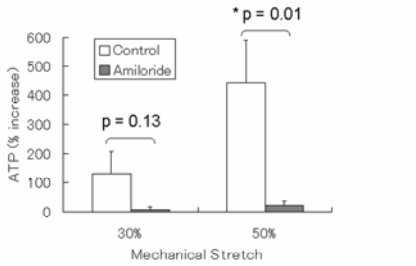

Fig 1.

Interpretation of results

We demonstrated that stretch evokes ATP release from the rat urinary bladder strips. This ATP release is mostly (over 90 %) from the epithelium, because the bladder strips without epithelium released much less ATP than whole layer strips. This is consistent with previous reports obtained from human being and swine bladder strips (2).

A mechanosensitive molecule in the urothelium is supposed to sense the stretch, then being involved in the stretch-evoked ATP release. We previously demonstrated that 1 mM amiloride, an antagonist of ENaC, increases the bladder capacity. Thus, ENaC expressed in the urothelium is a good candidate for mechanosensor in bladder afferent pathway. The present study indicated that amiloride inhibits the stretch-evoked ATP release from epithelium. The urothelium originated ATP plays an important role in afferent transduction in micturition reflex through $P2X_3$ receptors. Thus, amiloride-sensitive ENaC might be involved in the bladder afferent transduction mechanism by controlling the stretch-evoked ATP release from epithelium. However, it is still possible that amiloride might affect other mechanosensitive ion channels, which are also involved in this process.

Concluding message

Amiloride-sensitive ion channel, possibly ENaC, is involved in the stretch-evoked ATP release from rat urinary bladder epithelium, thus modulating the sensory transduction in the micturition reflex.

Reference

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(2) J Urol. 2004; 172(2): 744-747.

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