262

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MECHANISMS FOR STRETCH-INDUCED NON-NEURONAL ATP AND ACECHYLCHOLINE RELEASES FROM HUMAN BLDDER

Hypothesis / aims of study

Recently, it has been reported that the important role of acetylcholine (ACh) and adenosine triphosphate (ATP) released from non-neuronal source, especially from urothelium and suburothelial space, on pathogenesis of detrusor overactivity and overactive bladder (1). These seem to be the stimulating factors for afferent nerves resulting to increase in micturition reflex. There is a report demonstrated a high density of M_2 muscarinic receptor subtype in the human bladder urothelium (2). A recent report suggested that functional muscarinic or nicotinic receptors of urothelium contribute to non-neuronal ATP release in rats (3). However, the releasing mechanisms and relationship between two substances have not been fully evaluated. In the present study, we investigated the modulation mechanism for stretch-induced non-neuronal ATP release in human bladder

Study design, materials and methods

Human bladders were obtained from 15 patients (12 men and 3 women, 58 to 76 years old), who were undergoing cystectomy due to bladder carcinoma. Smooth muscle strips with and without urothelium were suspended in organ bath filled with Krebs-Henseleit solution. To obtain bladder strips without urothelium, the urothelium was carefully removed using scissors under microscope. Microdialysis probe was inserted into the strip, and Ringer solution was perfused into the probe at a constant flow rate of 2.0 µl/min, and dialysate was collected every 10 min in polyethylene tube. For ACh and ATP measurements, the dialysate was collected under 0 and 40 mN resting tension with TTX pre-treatment. The amount of ACh and ATP was measured by HPLC and luciferine-luciferase assay, respectively. Effects of various agents (botulinum toxin, amiloride, calcium ionophore, caffeine, muscarinic receptor antagonists and IP_3 receptor antagonist) on stretch-induced non-neuronal ACh and ATP releases were evaluated.

Results

In human bladder strips without urothelium, stretch-induced non-neuronal ACh and ATP releases were observed. However, the releases were about 10 to 15 % of those from strips with urothelium. Both releases from strips with urothelium were significantly inhibited in Ca^{2+} free medium by 50 to 65 %. However, the further addition of Ca^{2+} chelator (EGTA: 0.1 mM) resulted in a complete block of both releases. Calcium ionophore (A23187: 10 µM) significantly increased ATP release, and caffeine (10 mM), which depletes Ca^{2+} in intracellular store, significantly decreased the release. Treatment with botulinum toxin also caused significant reductions in both ACh and ATP releases. Treatment with amiloride (1 mM) decreased non-neuronal ATP, but it did not have a significant effect on non-neuronal ACh release. Atropine (0.001-1.0 µM) caused 72% reduction in stretch-induced non-neuronal ATP releases. Both methoctramine (M_2 selective antagonist: 0.01-1.0 µM) and 4-DAMP (M_3 selective antagonist: 0.01-10 µM) significantly inhibited the release. However, M_1 selective antagonist (pirenzepine: 0.01-10µM) did not have significant effects on the release. Furthermore, 2-aminoethoxdiphenyl borate (20 µM), an IP_3 receptor blocker, significantly inhibited non-neuronal ATP release.

Interpretation of results

The present data demonstrated that bladder urothelium/suburothelium main source of stretch-induced non-neuronal ACh and ATP releases. In the present experiment, Ca^{2}-free medium, Ca^{2+} chelator, Ca^{2+} ionophore, IP_3 receptor blocker and caffeine significantly inhibited the non-neuronal ACh and ATP releases. In addition, botulinum toxin, inhibitor of vesicular exocytosis or trafficking, significantly inhibited both releases. The data suggest that non-neuronal ACh and ATP release from human urothelium occur in part by Ca^{2}-dependent vesicular exocytotic process that involves an IP_3 signalling pathway. The present experiments also have demonstrated that muscarinic receptors contribute to the stretch-induced non-neuronal ATP release in human bladder. Significant inhibitions of non-neuronal ATP release by methoctramine and 4-DAMP suggest that stimulation of M_2 and M_3 receptor subtype by non-neuronal ACh partly contribute to non-neuronal ATP release.

Concluding message

The present data suggest that both intra- and extra-cellular Ca^{2+} contribute to stretch-induced non-neuronal ACh and ATP releases, and that vesicular exocytosis may be related to stretch-induced both releases in human bladder. Furthermore, non-neuronal ACh may stimulate non-neuronal ATP release through M_2 and M_3 receptor subtypes.

References

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