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Maeda Y[1], Yoshida M[1], Inadome A[1], Satoji Y[1], Murakami S[1], Masunaga K[1], Sugiyama Y[1], Nagata T[1], Ueda S[1]

1. Graduate School of Medical Sciences, Kumamoto University, Urology

NEURONAL AND NON-NEURONAL ACETYLCHOLINE AND ATP RELEASES IN RATS WITH SPINAL CORD INJURY

Hypothesis / aims of study

Spinal cord injury leads to alteration in bladder function. 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 /or suburothelial cells, on bladder dysfunction in pathological conditions. In the present study, we investigated the relationship between ACh and ATP releases from neuronal and non-neuronal origins and bladder function in rats with spinal cord injury.

Study design, materials and methods

Spinal cord was transected at the level of Th8-9 in female Sprague-Dawley rats. Sham-operated rat were used as a control. The experiments were performed 10 weeks after surgery. Urinary volume and micturition frequency was measured for 3 days in individual cage. Under anesthesia, small tube was implanted into femoral vein and bladder dome for filling cystometry. Cystometrograms were performed using constant infusion (0.05 ml/min) of saline into the bladder to elicit voiding. Saline voided from the urethral meatus was collected and measured to determine the voided volume. After cystometry, rats were sacrificed, and bladders were obtained. Smooth muscle strip was suspended in organ bath filled with Krebs-Henseleit solution, and tension development was recorded. Microdialysis probe was inserted into the strip with and without urothelium, and Ringer solution was perfused into the probe at a constant flow rate of 2.0 µl/min. Dialysate was collected during EFS (supramaximal voltage, 0.3 msec duration, 2.5 - 40 Hz and 3 sec train) and during muscle strip stretch (0 – 40 mN resting tension). EFS-induced releases were considered as neuronal releases. The stretch-induced tetrodotoxin (TTX)-insensitive releases were considered as releases from non-neuronal origin. The amount of ACh and ATP in the dialysate fraction was measured by HPLC-ECD and luciferine-luciferase assay, respectively.

Results

The mean weight of urinary bladders was significantly higher in SCI rats than in controls, while the mean body weight was not significantly different between both groups. In spinal rats, micturition frequency, micturition pressure, micturition threshold pressure and the number and amplitude of non-voided contractions were significantly higher, as compared to the control rats. Micturition volume and residual urine in spinal rats was significantly decreased and increased, respectively. EFS caused frequency-dependent increases in ACh and ATP (neuronal releases) in bladder smooth muscle strips in both groups. Both neuronal ACh and ATP releases from bladder strip in spinal rats were significantly lower than that from controls. In spinal rats, the decrease in non-neuronal ATP release was greater than that of ACh. Stretch of smooth muscle strip with urothelium caused tension-dependent increases in both ACh and ATP releases. The releases did not have effects with TTX (10^{-6} M) treatment. However, removal of the urothelium and/or suburothelial tissue caused about 85% reductions of both releases. Both ACh and ATP releases in spinal rats were significantly higher than that from control rats. In spinal rats, stretch-induced increase in ATP release was significantly greater than that of ATP.

Interpretation of results

In the present study, removal of urothelium caused significant reduction of non-neuronal ACh and ATP. The data imply that urothelium and suburothelial cells are main source of stretch-induced both releases. In the spinal rats, decrease in neuronal ATP release was greater than that of neuronal ACh release. In addition, increase in non-neuronal-ATP release was significantly higher than that of non-neuronal ACh. The data suggest that neuronal and non-neuronal ATP have significant contribution to bladder smooth muscle function in spinal rats, as compared with ACh.

Concluding message

In the present study, spinal cord injury caused changes in neuronal and non-neuronal ACh and ATP releases from bladder strips. It is suggested that neuronal and non-neuronal ATP have significant role on bladder dysfunction and detrusor overactivity in spinal rats, and that urothelium and suburothelial cells may contribute to the pathogenesis of the bladder dysfunction.

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