

## NEURONAL AND NON-NEURONAL ACETYLCHOLINE AND ATP RELEASES IN PARTIAL OUTLET OBSTRUCTION IN RATS

### Hypothesis / aims of study

Partial bladder outlet obstruction (BOO) 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, 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 BOO.

### Study design, materials and methods

BOO was performed by ligating the proximal urethra over 1mm catheter in female SD rats. Sham operated rats were served as controls. Rats were sacrificed 2 weeks and 3 months after introducing BOO. 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  $\mu$ l/min. The contractile responses induced by carbachol (0.01 $\mu$ -1mM), ATP (10 $\mu$ M-10mM), KCl (80 mM) and electrical field stimulation (EFS; supramaximal voltage, 0.3 msec duration, 2.5 - 40 Hz and 3 sec train) were evaluated using strips without urothelium. Using microdialysis technique, microdialysis probe was inserted into the strip, then the dialysate was collected during EFS (neuronal) and under TTX pre-treatment (non-neuronal) for measurements of ACh and ATP. Neuronal and non-neuronal releases were measured using strips without urothelium and with urothelium, respectively. The amount of ACh and ATP in the dialysate fraction was measured by HPLC and luciferine-luciferase assay, respectively. In addition, isolated bladder specimens were immunohistochemically stained for rabbit polyclonal S-100 protein and CGRP antibodies in both groups.

### Results

There were not significant changes in KCl-induced contractile response through the experimental period in both groups. Carbachol-induced contraction did not change until 3 months after introducing BOO. There was significant increase in ATP-induced contraction at 2 weeks and 3 months, as compared with each control group. EFS-induced contractions in bladder strips of BOO rats at 3 months was significantly increased, as compared with the control rats. Neuronal ACh and ATP releases from bladder strip were not significantly different between groups until 2 weeks. However, in 3 months after introducing BOO, both ACh and ATP releases were significantly lower in BOO rats than that in the control (Fig. 1). Non-neuronal ACh release was significantly increased in 3 month, and non-neuronal ATP was significantly increased in 2 weeks and 3 month, as compared with control rats. The increase rate of non-neuronal ATP was significantly higher than that of non-neuronal ACh (Fig.2). S-100 protein and CGRP positive neurons were observed in smooth muscle layers and suburothelial space, respectively. Density of S-100 protein positive neurons significantly decreased in 2 weeks and 3 months, however, the density of CGRP positive neurons significantly increased in 3 months, as compared with the control rats.

### Interpretation of results

The present study demonstrated that neuronal and non-neuronal ACh and ATP releases decreased and increased in BOO rats, respectively. In the immunohistochemical staining, decrease in the density of S-100 protein positive neurons suggest that partial denervation of motor neurons, and increase in the density CGRP positive neurons may imply the increased activity of afferent C neurons, respectively.

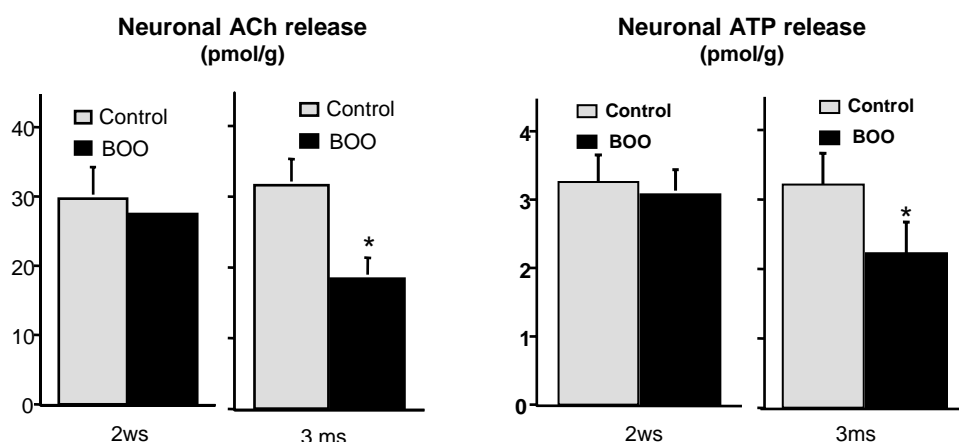


Fig.1 Neuronal ACh and ATP releases in BOO and control rats

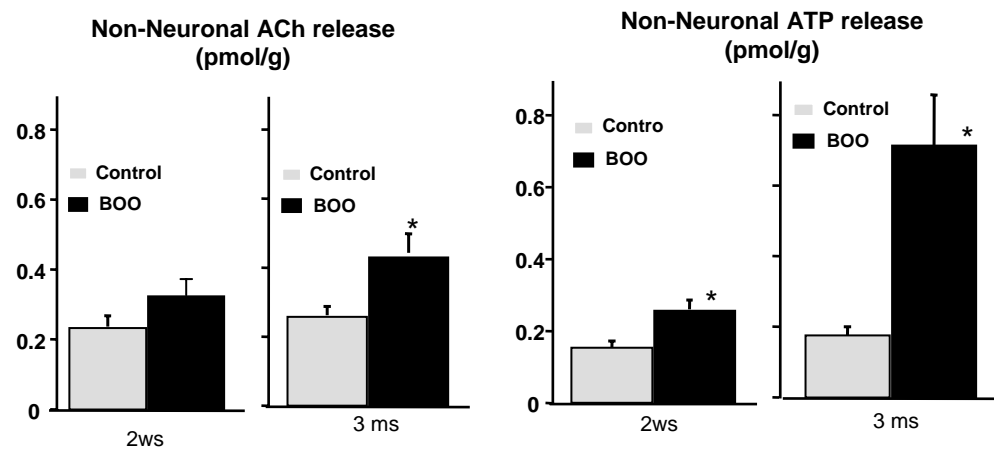


Fig.2 Non-neuronal ACh and ATP releases in BOO and control rats

#### Concluding message

The present data show that the decreased density of S-100 positive neuron may be related to the gradual decrease in neuronal ACh and ATP releases from the bladder strips of BOO rats. The denervation super-sensitivity may contribute to the increased ATP- and EFS-induced contractions. The significant increases in non-neuronal ATP and ACh releases were observed in bladder strips of BOO rats in 3 months. The data and the increased density of CGRP positive neurons suggest that the activation of afferent pathways may also contribute to detrusor overactivity in BOO rats.

**FUNDING:**

**NONE**

**DISCLOSURES:**

**NONE**

**ANIMAL SUBJECTS:**

This study followed the guidelines for care and use of laboratory animals and was approved by The ethics committee of Kumamoto University