

## EXPRESSION OF LARGE CONDUCTANCE, VOLTAGE- AND CA<sup>2+</sup>-ACTIVATED K<sup>+</sup> (MAXI K) CHANNEL IN HUMAN URINARY BLADDER: IDENTIFICATION OF MAXI K CHANNEL SUBUNITS AND DIFFERENCES BETWEEN NORMAL AND BLADDER OUTLET OBSTRUCTION.

### Hypothesis / aims of study

Large conductance, voltage- and Ca<sup>2+</sup>-activated K<sup>+</sup> (Maxi K) channels are thought to have a particularly prominent role to regulate urinary bladder smooth muscle excitability and contractility. The activation of Maxi K channels is supposed to contribute to the relaxation of the detrusor, and it is reported that the Maxi K channel opener suppressed the micturition reflex by decreasing afferent pelvic nerve activity. Therefore, Maxi K channels are candidate targets for overactive bladder therapy.

The present study aims to investigate the expression pattern of Maxi K channel in the human urinary bladder (the mucosal layer and the detrusor), how it changes in association with bladder outlet obstruction (BOO).

### Study design, materials and methods

Human bladders were obtained from 7 patients without prostatic enlargement and lower urinary tract symptoms (control group) who were undergoing total cystectomy due to bladder carcinoma, and 4 patients with BOO verified by urodynamic study (BOO group) who were performed suprapubic prostatectomy. The mucosal layer was peeled away from the detrusor, and total RNA was isolated from each specimen. A real-time RT-PCR was used to quantify the expressions of each Maxi K channel subunit ( $\alpha$ ,  $\beta$ 1-4) gene in the mucosa and the detrusor of human bladder. The expression and location of Maxi K channel proteins was examined using immunohistochemistry (IHC) with affinity-purified anti-Maxi K $\alpha$  and anti-Maxi K $\beta$  antibodies.

### Results

RT-PCR analysis revealed the presence of  $\alpha$ - and  $\beta$ 1-subunit genes of Maxi K channel in both the mucosal layer and the detrusor, while  $\beta$ 2-4 subunit genes were substantially absent (Figure 1). The expression levels of  $\alpha$ 1- and  $\beta$ 1-subunit mRNA in the detrusor layer were significantly higher than those in the mucosal layer. The expressions of both subunit genes in mucosal layer significantly decreased in BOO bladders compared with control, and those in the detrusor also showed a significant decrease in BOO (Table 1). IHC staining of Maxi K- $\alpha$  and Maxi K- $\beta$  proteins were observed in both the submucosal and the muscle regions of the bladder. The staining density of these proteins in the detrusor muscle was obviously low in BOO bladders, and that in the mucosal layer and the submucosal space showed some differences between control and BOO bladders.

Figure 1. Maxi K channel subunits mRNA expression in the mucosal layer and the detrusor

RT-PCR products of Maxi K channel  $\alpha$ - and  $\beta$ 1-4 subunits in the human urinary bladder mucosal layer (A) and detrusor (B) were shown.  $\beta$ -actin was used as a control.

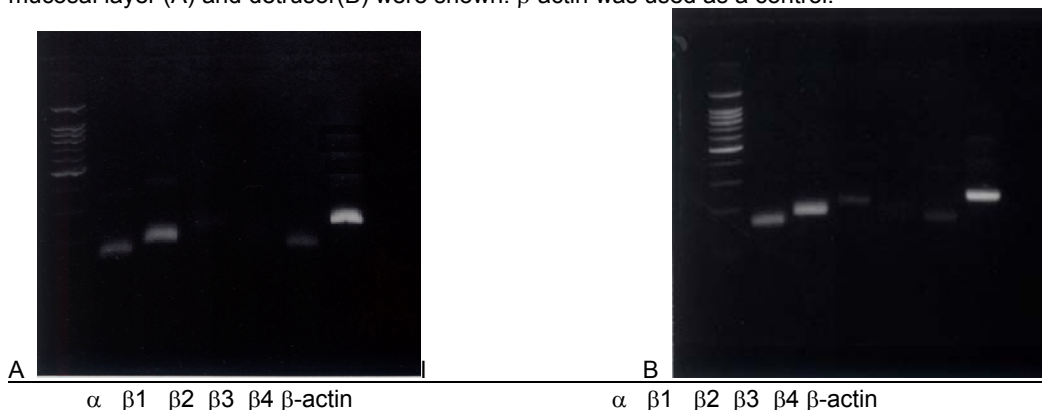
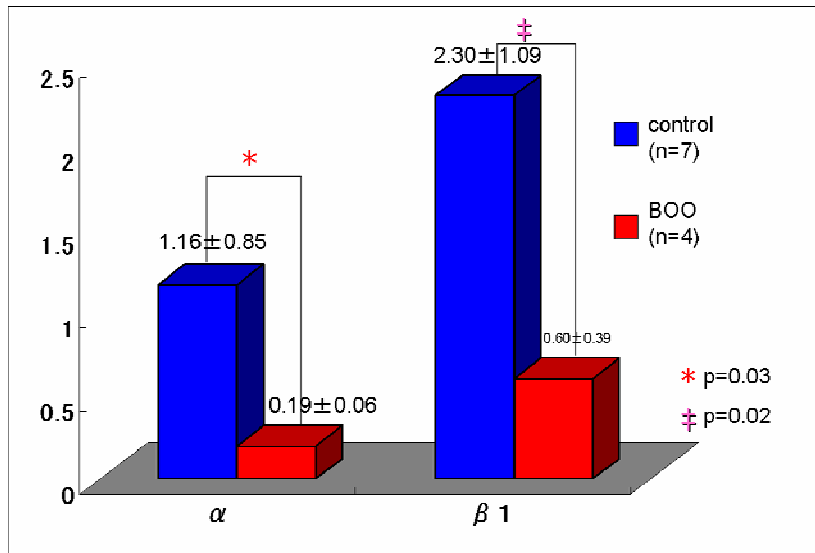


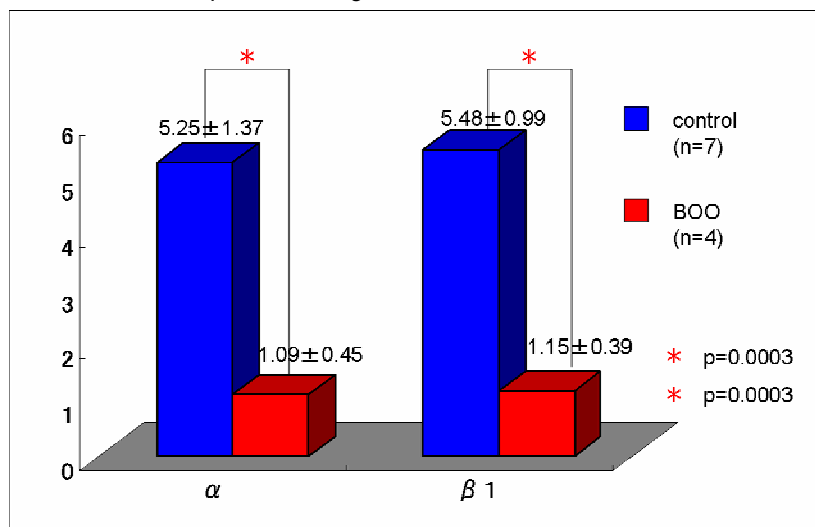
Table 1, relative product strengths of Maxi K- $\alpha$  and - $\beta$ 1 subunit mRNA determined by densitometry (density of product/density of  $\beta$ -actin) in control and BOO bladders

The expressions of both subunit genes in mucosal layer (A) and the detrusor (B) significantly decreased in BOO bladders compared with control.

A, relative RT-PCR product strengths of Maxi K subunit mRNA in mucosal layer



B, relative RT-PCR product strengths of Maxi K subunit mRNA in the detrusor



#### interpretation of results

Opening of Maxi K channels regulates membrane potential and relaxes bladder smooth muscle. So, Maxi K channels are thought to have an important role in the regulating urinary bladder function, and its dysfunction may lead to overactive bladder and urinary incontinence.

There are several reports on the utility of Maxi K channels as potential drug targets for modulating urinary bladder function. In this study, we have demonstrated the existence of Maxi K- $\alpha$  and - $\beta 1$  subunits in human urinary bladder (both in the mucosal layer and the detrusor) by using a combination of genetic and histochemical approaches. The expression level of Maxi K channels in the detrusor muscle and the mucosal layer decreased in BOO bladders compared with controls. These results suggest that Maxi K channels have a pivotal role in the mechanisms of overactive bladder induced by bladder outlet obstruction.

#### Concluding message

Our study provides the evidence that Maxi K- $\alpha$  and - $\beta 1$  subunits are presented in human urinary bladder, and indicates that the decrease of these Maxi K channel subunits participate in inducing overactive bladder occurred by bladder outlet obstruction.

**FUNDING:** NONE

**DISCLOSURES:** NONE

**HUMAN SUBJECTS:** This study was approved by the The Ethical committee in University of Yamanashi and followed the Declaration of Helsinki Informed consent was obtained from the patients.