RELATIVE CONTRIBUTION OF RHOA/RHO-KINASE PATHWAY AND PKC/CPI-17 PATHWAY TO MUSCARINIC RECEPTOR-MEDIATED DETRUSOR CONTRACTION IN THE RAT OBSTRUCTED BLADDER

Hypothesis / aims of study
In the smooth muscle, when agonists bind to G-protein coupled receptors, the concentration of intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) increases temporarily, which causes smooth muscle contraction. However, after [Ca\(^{2+}\)]\(_i\) returns to its basal level, smooth muscle remains contracted. This mechanism that sustains smooth muscle contraction independently of Ca\(^{2+}\) is referred to as Ca\(^{2+}\) sensitization. In detrusor smooth muscle, two major pathways, a RhoA/Rho-kinase (ROK) pathway and a protein kinase C (PKC)/PKC-potentiated protein phosphatase-1 inhibitor protein (CPI-17) pathway, are involved in muscarinic receptor (MR)-mediated Ca\(^{2+}\) sensitization. Although attention has recently focused on the role of ROK in bladder dysfunction secondary to bladder outlet obstruction (BOO), the role of PKC/CPI-17 pathway in this pathologic condition has remained to be elucidated. Thus, the present study was undertaken to investigate whether BOO alters the relative importance of RhoA/ROK and PKC/CPI-17 pathways in Ca\(^{2+}\) sensitization induced by MR signalling.

Study design, materials and methods
Partial BOO was obtained by the placement of a silk ligature at the bladder neck, and control animals underwent a sham procedure. Four weeks later, the bladder was removed through an abdominal incision. Each longitudinal bladder muscle strip (approximately: 10 mm x 3 mm) was prepared and suspended in a 25 ml organ bath containing Krebs solution. Functional studies were performed on detrusor smooth muscle pre-contracted with 1μM Carbachol (Cch). Concentration-response curves for ROK inhibitor (Y-27632) and PKC inhibitor (GF-109203X) were obtained by cumulative addition of each inhibitor. In the biochemical studies, SDS-PAGE and Western blotting were performed by using the samples extracted from the bladder smooth muscle. Expression of ROK isoforms (ROK alpha and ROK beta) and CPI-17 were measured with anti-ROK alpha, anti-ROK beta, and anti-CPI-17 antibodies.

Results
Cch (1μM)-induced contraction of detrusor muscle from both control and obstructed bladder showed an initial phasic contraction followed by a sustained tonic contraction. The magnitude of sustained contraction in the obstructed bladder was significantly greater than that in the control bladder. The effects of ROK inhibitor (Y-27632) and PKC inhibitor (GF-109203X) on the sustained contraction induced by 1μM Cch were determined. Both Y-27632 and GF-109203X at concentrations from 1 to 30μM significantly attenuated the Cch-induced contraction of detrusor muscle from the control group (Fig.1A). This inhibitory effect of Y-27632 was slightly but significantly greater than that of GF-109203X at the above concentrations in control bladders. However, in obstructed bladders, the relaxation effect of Y-27632 increased while that of GF-109203X decreased and there was a significant difference in relaxation effect at concentrations from 0.1 to 30μM between Y-27632 and GF-109203X (Fig.1B). As compared to the control, the
expression of ROK alpha and ROK beta significantly increased (2.08±0.30 fold and 3.30±1.21 fold, respectively) while the expression of CPI-17 significantly decreased (0.63±0.15 fold) in the obstructed bladder (Fig.2).

Interpretation of results
In normal rat bladder, both RhoA/ROK and PKC/CPI-17 pathways were shown to be involved in the MR-mediated sustained contraction of detrusor muscle via Ca²⁺ sensitization. In the obstructed bladder, however, an increase in inhibitory effect of Y-27632 and a decrease in inhibitory effect of GF-109203X suggest that the upregulation of RhoA/ROK pathway and the downregulation of PKC/CPI-17 pathway may occur. Supporting this, this study also demonstrated that the expression level of ROK isoforms increased while that of CPI-17 decreased in the obstructed bladder. Thus, in the two pathways involved in MR-mediated Ca²⁺ sensitization, the RhoA/ROK pathway becomes dominant to sustain detrusor contraction in the obstructed bladder.

Concluding message
MR-mediated Ca²⁺ sensitization pathway is shifted to the RhoA/ROK pathway dominance in the obstructed bladder.

References
1 Br J Pharmacol (2006) 148; 376-384