71

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OBSTRUCTION ALTERS MUSCARINIC RECEPTOR-ACTIVATED RHOA/RHO-KINASE PATHWAY IN THE URINARY BLADDER OF THE RAT

Hypothesis / aims of study

It is widely accepted that the concentration of intracellular Ca^{2+} ($[Ca^{2+}]_i$) is a primary determinant of smooth muscle contraction. In bladder smooth muscle, when agonists bind to muscarinic receptors (MR_s), $[Ca^{2+}]_i$ increases temporarily, which causes calmodulin-mediated activation of myosin light chain (MLC) kinase. This kinase, in turn, phosphorylates MLC and, thereby, induces 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. One main pathway inducing Ca^{2+} sensitization is known to involve Rho-kinase (ROK), which is activated by RhoA via G-protein coupled receptors. Recently, alteration has focused on a role of ROK in bladder dysfunction secondary to bladder outlet obstruction (BOO). Although the increased expression of ROK has been demonstrated in detrusor muscle from rabbit obstructed bladders[1], a change in MR-activated RhoA/ROK pathway remains to be elucidated. Since the bladder contraction involved in voiding is mediated predominantly by MR_s, information on Ca^{2+} sensitization mediated by MR signalling would provide further understanding of detrusor dysfunction following BOO. Thus, the present study was undertaken to determine whether BOO alters MR-activated RhoA/ROK pathway in detrusor muscle of the rat.

Study design, materials and methods

Partial bladder outlet obstruction (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. Longitudinal bladder muscle strips (approximately: 10 mm x 3 mm) were prepared and suspended in a 25 ml organ bath containing Krebs solution. In the biochemical studies, SDS-PAGE and Western blotting were performed by using the samples extracted from the bladder smooth muscle. Expression of RhoA and Both ROK isoforms (ROK alpha and ROK beta) were measured with anti-RhoA, anti-ROK alpha, and anti-ROK beta antibodies. In addition, phosphorylation of MLC20 at serine[19] was measured by Western blotting with anti-MLC20 and anti-Ser^{19} phosphorylated MLC20 antibodies. The ratio of MLC20 phosphorylated at Ser^{19} to total MLC20 was calculated. Functional studies were performed on detrusor smooth muscle pre-contracted with 10^{-6} M Carbachol (Cch). Concentration-response curves for ROK inhibitors (Y-27632 and fasudil) were obtained by cumulative addition of each substance.

Results

Obstructed bladders were approximately 4 times heavier than sham bladders ($p<0.01$), and there was no marked deterioration in contractility. The contractile response to Cch clearly increased in the BOO rats especially at 30 minutes after exposed to Cch (sustained phase) ($p<0.05$). In addition, MLC phosphorylation level at the sustained phase from the BOO group was significantly higher than in the sham group ($p<0.05$). Expression of RhoA, ROK alpha, and ROK beta were significantly increased 1.44 ± 0.05 fold ($p<0.01$), 1.95 ± 0.28 fold ($p<0.01$) and 3.11 ± 1.04 fold ($p<0.05$), respectively, in the BOO rat compared to the sham (Fig.1). The relaxing effect of Y-27632 on the pre-contracted bladder strip from the BOO rat was stronger than on strips from the sham rat (Fig.2A), and statistically significant at 10^{-7} M, $3x10^{-7}$ M, 10^{-6} M, and $3x10^{-6}$ M, (all $p<0.01$). The results for fasudil were similar to those obtained with Y-27632 (Fig.2B).

Interpretation of results

The detrusor smooth muscle from BOO rats contracted more strongly and longer in response to Cch than that from the sham. RhoA/ROK pathway seems to be involved in this enhanced MR-mediated contraction of the obstructed bladder. This is supported by the increased expression of RhoA and ROK isoforms in BOO rat. Consistent with these observations, the inhibitory effect of ROK inhibitor (Y-27632 and fasudil) on Cch-induced contraction increases in detrusor muscle from BOO rats. The effect of ROK inhibitor enhanced under pathological conditions, and from the view of clinical application, this may be beneficial.

Concluding message

The finding that an enhanced MR-mediated RhoA/ROK pathway points to an adaptation of the detrusor to facilitate force maintenance for a longer period of time in an attempt to empty the bladder against the obstruction. In addition, if ROK is up-regulated in the human bladder in BPH, ROK would be an interesting target for the treatment of obstructed bladder symptoms associated with BPH.

References

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Fig.1 Expression of RhoA and ROK isoforms at the protein level

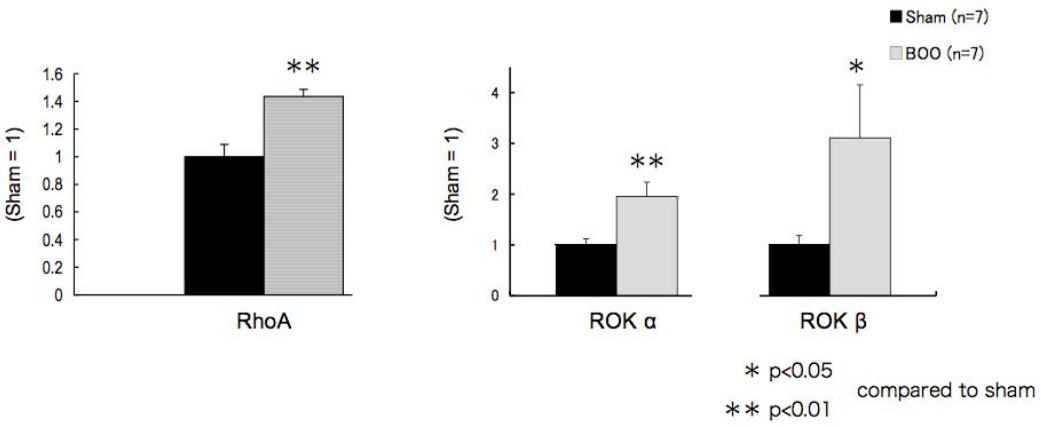
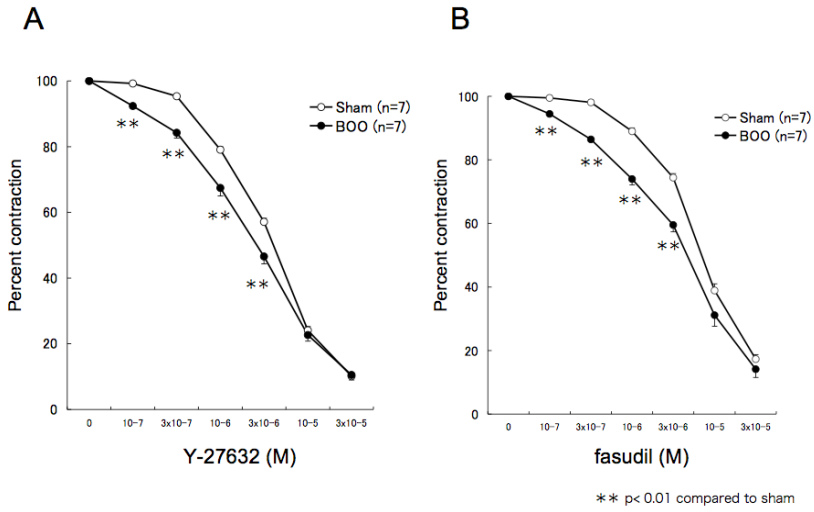


Fig.2 Relaxing effects of ROK inhibitors



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