OBSTRUCTION ENHANCES RHO-KINASE PATHWAY IN CARBACHOL-INDUCED CA2+ SENSITIZATION IN ALPHA-TOXIN PERMEABILIZED GUINEA-PIG DETRUSOR SMOOTH MUSCLE

Hypothesis / aims of study
Cell permeabilization using α-toxin from Staphylococcus aureus has been used successfully in guinea-pig detrusor smooth muscle preparations which show a more sustained maintenance of force of the smooth muscle contractile system and preserves the receptor-effector pathways while allowing direct evaluation of changes in Ca2+ sensitivity.1 Rho kinase (ROK) pathway was reported to have a role in carbachol (CCh)-induced Ca2+ sensitization in β-escin skinned detrusor smooth muscle of guinea-pig.2 Since the increased expression of ROK was reported in detrusor smooth muscle of rabbit following bladder outlet obstruction (BOO),3 the role of ROK pathway in CCh-induced Ca2+ sensitization in detrusor smooth muscle following BOO remains to be elucidated. The aim of this study was to investigate the role of ROK pathway in CCh-induced Ca2+ sensitization in α-toxin permeabilized smooth muscle fiber obtained from guinea-pig detrusor smooth muscle following BOO.

Study design, materials and methods
Partial bladder outlet obstruction (BOO) was created by the placement of silver jump rings with internal diameter 2.2 mm on bladder neck through lower midline incision of the abdomen. Sham operated guinea-pig underwent surgery following a similar protocol without the application of the rings. The guinea-pigs were sacrificed after eight weeks and the bladder was removed and placed in oxygenated Krebs solution. The mucosa and connective tissues were removed from the bladders under a dissecting microscope. The smooth muscle were dissected into small strips (300–400 μM in diameter, 2-3 mm in length). The strips were mounted between 2 tungsten wires, of which 1 was fixed, while the other was attached to a force transducer in a Perspex block with 100 μl physiological salt solution buffered by HEPES-Tris under a resting of 100 mg. Solution changes were made by moving the Perspex block. Permeabilization of the smooth muscle strips was done in relaxing solution composed of 100 mM potassium methansulphonate, 2.2 mM Na2 adenosine triphosphate, 3.38 mM MgCl2, 10 mM ethyleneglycol-bis-(b-aminoethyl-ether)-N',N',N',N'-tetra acetic acid (EGTA), 10 mM creatinine phosphate and 20 mM tris-maleate (pH 6.8) containing 0.2 mg/ml Staphylococcus aureus α-toxin for 1-2 hours. The effect of ROK inhibitor (Y-27632) on CCh-induced Ca2+ sensitization was studied by the application of 5μM Y-27632 during sustained contraction induced by 1 μM Ca2+ added with 1μM calmodulin (CaM), 100mM GTP and 10 μM CCh (fig.1). The experiment were carried out with the cyclopiazonic acid (CPA; 1μM) present in all solutions after permeabilization and performed at room temperature. The effect of Y-27632 are expressed as % of the response to sustained contraction induced by 1 μM Ca2+ added with 1μM calmodulin (CaM), 100mM GTP and 10 μM CCh. All data are expressed as the mean ± s.e.m. of n experiments. Statistical analyses were carried out by using Student t-test for comparing two groups (BOO and sham). P<0.05 were accepted as statistically significant.

Results
The application of 5μM Y-27632 during sustained contraction induced by 1 μM Ca2+ added with 1μM CaM, 100 μM GTP and 10 μM CCh at constant [Ca2+], caused a relaxation of 43.7 % ± 2.5 % in BOO group (n=4) and 21.5% ± 1.5 % in sham group (n=4) respectively. The difference of relaxation effect of Y-27632 between two groups was statistically significant (p = 0.017, fig.2).

Interpretation of results
The evidence of this study which demonstrated a significant higher inhibition effect of Y-27632 in BOO guinea-pig revealed that the effect of Y-27632 was augmented by obstruction. This study suggest that obstruction enhanced the ROK pathway in guinea-pig detrusor smooth muscle.

Concluding message
The results of this study indicate that obstruction enhances ROK pathway in guinea pig detrusor smooth muscle. This pathway might be important as a target in the treatment of lower urinary tract symptoms related to BOO.

Fig. 1. CCh-induced calcium sensitization in guinea-pig bladder with Ca2+ clamped at pCa 6. Consecutive increases in tension in guinea-pig bladder were induced by 1μM CaM, 100μM GTP, and 10μM CCh. Y-27632 (5μM) added in the presence of CCh reversed the contraction by this muscarinic receptor agonist.
Fig. 2. The inhibition effect of Y-27632 (5μM) on CCh-induced Ca²⁺ sensitization at pCa 6 in guinea pig detrusor smooth muscle of sham (n=4) and BOO group (n=4).

References

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