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INVOLVEMENT OF STRETCH-INDUCED RHO-KINASE ACTIVATION AND BLADDER TONE IN THE STORAGE PHASE.

Hypothesis / aims of study

Rho-kinase (ROK) is known to be a part of the pathways contributing to the phenomenon of Ca^{2+} sensitization, which is involved in the regulation of bladder smooth muscle contraction.

The ROK inhibitor attenuates bladder contractions induced by G-protein coupled receptor agonists. In addition to affecting contraction, the ROK inhibitor causes a significant decrease in resting tension of the detrusor muscle strip in the absence of agonist stimulation. This suggests that ROK may play a role in the maintenance of bladder tone during the storage phase (where there is stretch stimulation, but no agonist stimulation), thereby affecting bladder compliance. However, data obtained from the muscle strip study may not be directly applicable to the pressure-volume relationship of the intact bladder. This study was undertaken to investigate whether stretch activate ROK in bladder muscle by Western blotting. Furthermore, using an in vitro whole bladder model, we investigated whether ROK inhibitor (HA1077) can increase bladder compliance.

Study design, materials and methods

Study 1: The rat bladder was removed for muscle strip studies to evaluate the effects of the ROK inhibitors, Y-27632 and HA-1077 on resting tension and KCl-induced tonic contractions (independent of G-protein coupled pathway). Study 2: we used the samples extracted from the bladder muscle strips in Krebs buffer. Western blotting was used to determine stretch-induced ROK activation in bladder muscle strips by measuring phosphorylation of MYPT1 (myosin phosphatase targeting subunit). Furthermore, to investigate whether stretch-induced ROK activation is dependent of Ca^{2+}, we also use the samples extracted from the bladder muscle strips in Ca^{2+} -free Krebs buffer with thapsigargin, a potent inhibitor of the sarcoplasmic Ca^{2+} ATP-ase pump. Study 3: An in vitro whole bladder model was used to examine the effect of ROK inhibitor on bladder compliance during bladder filling.

Results

Study 1: Y-27632 and HA-1077 caused a concentration-dependent relaxation of the resting tension and KCl-induced tonic contraction of detrusor strip (Fig. 1). Study 2: Stretch increased the degree of MYPT1-p[Thr853] by nearly 1.5-fold above the basal level produced by tissues in a normal Krebs buffer and in Ca^{2+}-free solution. The ROK inhibitor Y-27632 abolished the increase produced by stretch and reduced the basal level of MYPT1-p[Thr853] to <50% in a normal Krebs buffer and in Ca^{2+}-free solution (Fig. 2). Study 3: When tested in a rat isolated whole bladder model, both 3μM and 10μM HA-1077 produced a significant increase in compliance compared to vehicle (Fig. 3)

Interpretation of results

The inhibitory effects of ROK inhibitors on resting tension and KCl-induced contraction of detrusor strips suggest that ROK pathway may be involved in the maintenance of bladder tone at the adequate tension, independent of G-protein coupled pathway. Our results of Western blot ting indicate that ROK is constitutively active, and that stretch further activates ROK. The present results of whole bladder study demonstrate that ROK inhibitor increases bladder compliance during bladder filling.

Concluding message

This study demonstrates that stretch activates ROK pathway, which contributes to the maintenance of bladder tone, thereby affecting bladder compliance. Therefore, it would appear that blockade of ROK may represent a useful therapeutic approach to over active bladder by increasing bladder compliance.

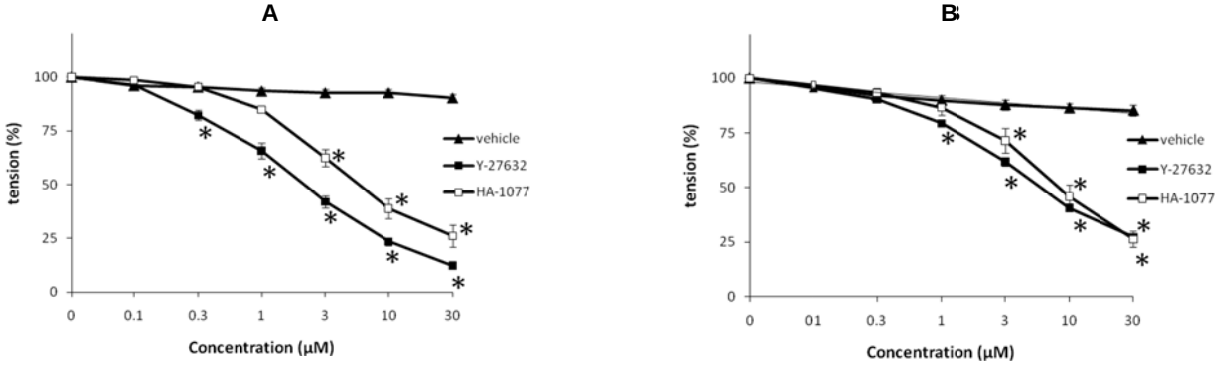

Fig. 1. The effects of ROK inhibitor, Y-1077 and HA-1077 on the bladder strip. **A:** Effects of ROK inhibitor on resting tension. **B:** Effects of ROK inhibitor on KCl-induced contraction. Values represent mean ± SEM (n=6). * P<0.05 compared to vehicle.

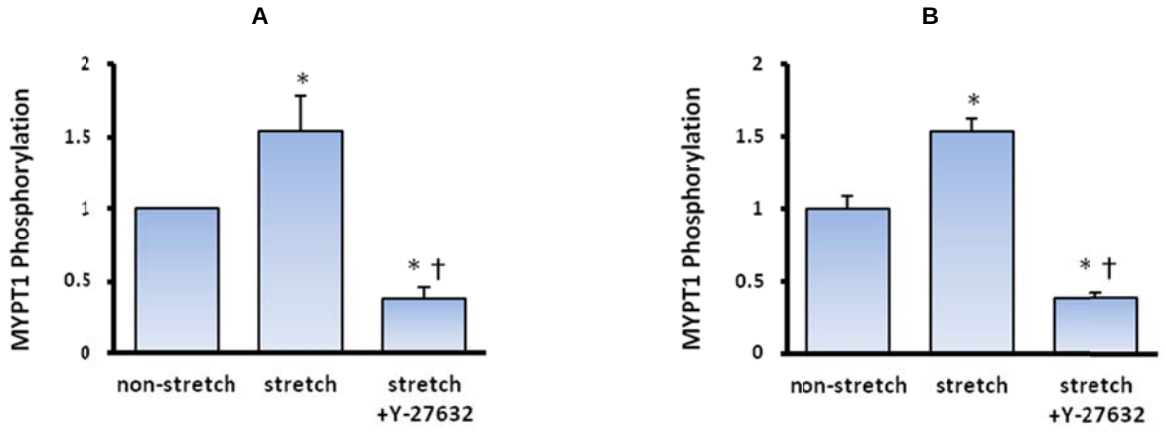

Fig. 2. Changes in MYPT1 phosphorylation at Thr853 by stretch and Y-27632. **A:** Average values of Western blots of MYPT1 phosphorylation in normal Krebs. **B:** Average values of Western blots of MYPT1 phosphorylation in Ca^{2+}-free Krebs with thapsigargin. Each value represents the mean ± SEM (n=6). * P<0.05 compared to non-stretch; † P<0.05 compared to stretch.

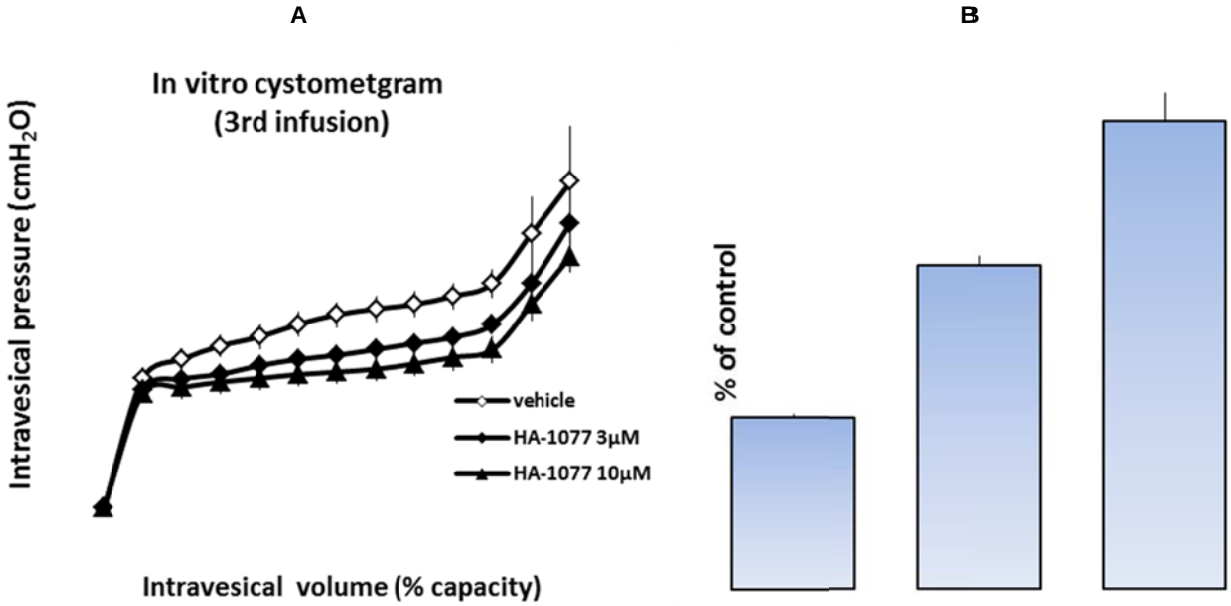

Fig. 3. In vitro whole bladder study. **A:** in vitro cystometgram. **B:** Effects of HA-1077 on bladder compliance. Each value represents the mean ± SEM (n=6). * P<0.05 compared to vehicle. ** P<0.01 compared to vehicle.

Specify source of funding or grant	**Japan Society for the Promotion of Science**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Fukushima medical university**