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Shiomi H[1], Shishido K[1], Takahashi N[2], Kawashima Y[1], Ogawa S[1], Yazaki J[1], Ishibashi K[1], Aikawa K[1], Yamaguchi O[1]
1. *Fukushima Medical Universitiy,* ***2.*** *Ohta General Hospital*

RHO-KINASE INHIBITOR INCREASES BLADDER COMPLIANCE IN AN IN VITRO RAT WHOLE BLADDER MODEL

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. HA-1077 is considered to be a specific ROK inhibitor and attenuates bladder contractions induced by G-protein coupled receptor agonists. In addition to affecting contraction, the ROK inhibitor (HA-1077) 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 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. In the present study, using an in vitro whole bladder model, we investigated whether ROK inhibitor (HA1077) can increase bladder compliance. Furthermore, this study also evaluated an inhibitory effect of HA-1077 on carbachol (Cch)-induced bladder contraction of the whole bladder model, which mimics detrusor contraction during voiding.

Study design, materials and methods

Male Sprague-Dawley rats were used in this study. Study 1: The bladder strips were mounted in organ baths containing warmed Krebs buffer. A resting tension of 1g was applied to the preparation at the beginning of the experiment, and a concentration-response curve was obtained by cumulative addition of HA-1077 (0.1-30μM) to the bathing fluid. Data were expressed as percent of maximal relaxation induced by 100μM papaverine. Study 2: The in vitro rat whole bladder models were used to examine the effects of HA-1077 on rat bladder compliance during filling. The ureters were ligated with 3-0 silk close to the bladder body and cut distally to the ties. The bladders were removed and cannulated via the urethra with PE50 tubing and mounted in 20ml organ baths containing warmed Krebs buffer. The bladders were infused with physiological saline at 0.05ml per minute and the intravesical pressures were recorded. This cystometgram consisted of the flat part of intravesical pressure (tonus phase) and the following ascending part (terminal phase). Bladder compliance was measured in the tonus phase. After a control infusion was performed, the bladders were incubated for 30 minutes with either HA1077 (3, 10μM) or vehicle. This was followed by a second infusion, and the changes of data were recorded. Study 3: We also investigated the effects of HA-1077 on carbachol-induced contractions of in vitro whole bladders. With the bladders filled to half-capacity, carbachol (10μM) was added to the bath for 5 minutes and intravesical pressures were monitored. After the bladders were washed out, tissues were then incubated with HA-1077 or vehicle for 30 minutes and carbachol was again added in the same manner. Then intravesical pressures were again monitored.

Results

Study 1: HA-1077 caused a concentration-dependent relaxation of the detrusor strip, and the changes were significant ($p<0.01$) at concentrations from 1μM to 30μM compared to vehicle (Figure1). Study 2: Both 3μM and 10μM HA-1077 produced a significant increase in compliance ($p<0.05$, $p<0.01$, respectively) compared to vehicle (Figure2, 3, Table1). Study 3: 3μM HA-1077 did not produce a significant inhibition of carbachol evoked bladder contraction ($p=0.4$); whereas 10μM HA-1077 produced a significant inhibition ($p<0.05$) compared to vehicle (Figure4).

Interpretation of results

This study using the in vitro whole bladder model demonstrates that ROK inhibitor (HA-1077) increases bladder compliance. HA-1077 (3μM and 10μM) caused a dose-dependent increase in bladder compliance by 83% and 165%, respectively; whereas these doses of HA-1077 decreased carbachol-induced contractions by only 5% and 18.3%, respectively. Therefore, ROK inhibitor at the low dose (3μM in this case) can increase bladder compliance without affecting detrusor contraction.

Concluding message

Our results suggest that ROK inhibitor can increase bladder compliance. Therefore, blockade of ROK may be useful for the treatment of lower urinary tract dysfunction, such as overactive bladder.

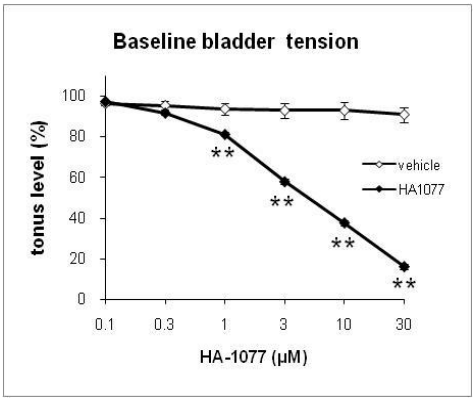

Figure1. Effects of HA-1077 on baseline bladder tension in rat urinary bladder strips. Each value represents the mean ± SEM (n=6).
Statistical analysis performed using unpared t test.
* $p<0.01$ vs. vehicle.

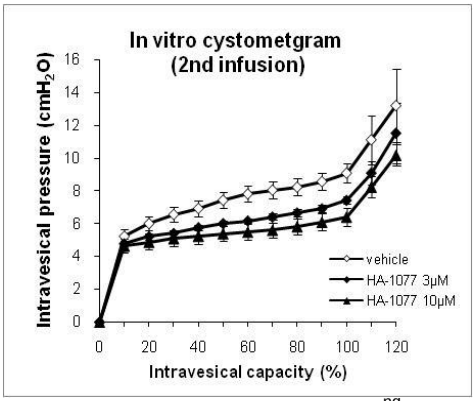

Figure2. In vitro cystometgrams. (2^{nd} infusion)
Each value represents the mean ± SEM (n=6).

Table1. Results of repeated in vitro cystometry before and after incubation with HA-1077

	Bladder compliance ($ml/cmH_2O \times 100$)	
	Control	Drug
vehicle	21.4 ± 1.68	20.7 ± 1.86
HA-1077 (3μM)	23.1 ± 3.48	40.0 ± 4.50*
HA-1077 (10μM)	25.5 ± 5.11	62.6 ± 8.21**

Each value represents the mean ± SEM (n=6).
Statistical analysis performed using pared t test.
* p<0.05, ** p<0.01 vs. control.

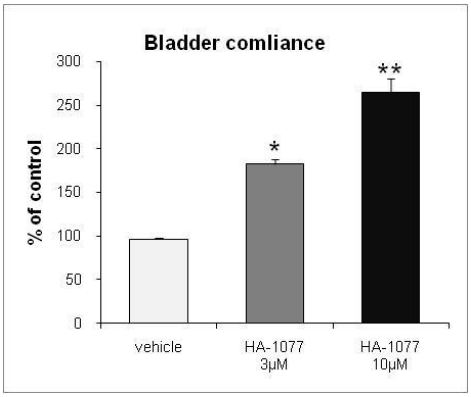

Figure3. Effects of HA-1077 on bladder compliance of in vitro whole bladders.
Each value represents the mean ± SEM (n=6).
Statistical analysis performed using one-way ANOVA followed by Dunett's post-test.
* p<0.05, ** p<0.01 vs. vehicle.

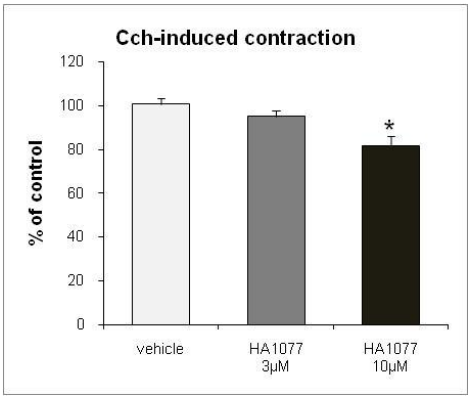

Figure4. Effects of HA-1077 on Cch-induced contraction of in vitro whole bladders.
Each value represents the mean ± SEM (n=6).
Statistical analysis performed using one-way ANOVA followed by Dunett's post-test.
* p<0.05 vs. vehicle.

References

1. Br J Pharmacol (2003)138; 757-766
2. J Urol (2003)169; 756-760
3. J Urol (1994)151; 496-502

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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	**the Animal Ethics Committee of Fukushima Medical University**