115

Yamanishi T[1], Yoshida K[1], Nakanishi K[1], Sakakibara R[2], Uchiyama Y[3]
***1.** Dokkyou Medical University, **2.** Toho University Sakura, **3.** Chiba University*

EXPRESSION OF RHOA MRNA AND ACTIVATED RHOA IN UROTHELIUM AND SMOOTH MUSCLE, AND EFFECTS OF A RHO-KINASE INHIBITOR ON CONTRACTION OF THE PORCINE URINARY BLADDER

Hypothesis / aims of study
By reorganizing the actin cytoskeleton, Rho, a small monomeric G-protein, and the Rho-associated serine-threonine protein kinase (ROCK) pathway have been reported to participate in various cellular functions, including smooth muscle contraction, cell adhesion, and cell migration. The Rho/ROCK pathway has been reported to have a role in the contraction of non-vascular smooth muscle at sites such as the bladder in rabbits by modifying the sensitivity of contractile and regulatory proteins to intracellular calcium ion concentration or $[Ca^{2+}]_i$, a phenomenon referred to as "Ca^{2+} sensitization". We investigated the expression of RhoA by real-time reverse transcription-polymerase chain reaction (RT-PCR), and the amount of activated RhoA by a RhoA activation assay, in the urothelium and smooth muscle of the pig bladder. As *in vitro* functional studies, the inhibitory effect of Y-27632 on tissue precontracted with KCl or carbachol, as well as the response to field electrical stimulation, were examined in porcine bladder tissues, comparing specimens with and without urothelium. In another study, cumulative concentration-response curves (CRCs) to carbachol were obtained with and without 3-10 μM Y-27632, and the effects of Y-27632 on carbachol-induced contraction of porcine urinary bladder smooth muscle with or without urothelium were assessed.

Study design, materials and methods
RhoA expression was studied by the real-time reverse transcriptase polymerase chain reaction (RT-PCR) and these activities were studied by Western blotting. In functional studies, the cumulative concentration-response curve (CRC) for Y-27632 (0.1 nM-100 μM) was examined using tissue strips precontracted with KCl and carbachol, and the response to electrical stimulation of bladder smooth muscle with and without urothelium was examined. In another study, the effects of 3-10 μM Y-27632 on CRC to carbachol in bladder tissue strips with or without urothelium were compared.

Results
The expression of RhoA mRNA (n=38) and the amount of activated RhoA (n=19) were greater in the urothelium than in the smooth muscle. For strips without mucosa, tension after administration of Y-27632 (1nM-100μM) was 76.5±7.8% (n=7) and 69.4±5.81% (n=6), respectively, of the value obtained after pre-contraction with KCl and carbachol. Maximum contraction (Emax) on CRC to carbachol after the administration of 10 and 30 μM Y-27632 (66.7±8.25% and 85.6±2.6%, respectively, of the control value) was significantly reduced (both $p<0.05$), (n=36). The pEC_{50} values obtained after administration of 3, 10, and 30 μM Y-27632 (5.33±0.12, 5.28±0.11, and 5.23±0.03, respectively) were significantly reduced (all $p<0.05$). The response to electrical stimulation at 50 Hz after administration of 3, 10, and 30 μM Y-27632 was 98.7±4.53%, 100.4±5.91%, and 73.1±34.5% of the initial value, respectively (n=7). For strips with intact mucosa, tension after administration of Y-27632 (1nM-100μM) was 58.2±5.3% (n=11) and 40.5±7.40% (n=6), respectively, of the value obtained after pre-contraction with KCl and carbachol. Emax on CRC to carbachol decreased significantly after the addition of 3, 10, and 30 μM Y-27632 (72.2±6.8%, 43.9±7.1%, and 25.0±5.5%, respectively, of the control value; $p<0.05$, $p<0.0001$, and $p<0.0001$, respectively) (n=20). The pEC_{50} values also decreased significantly after addition of 3 and 10 μM Y-27632 (4.68±0.08 and 5.19±0.15, respectively; both $p<0.05$). Administration of 3, 10, and 30 μM Y-27632 also decreased the response to electrical field stimulation at 50 Hz to 57.8±19.0%, 47.3±29.0%, and 33.8±15.0% of the initial value, respectively (n=11). The decrease of tension after administration of 3, 10, and 30 μM Y-27632 was concentration-dependent in tissue strips with intact mucosa, and was greater than in tissues without mucosa.

Interpretation of results
In the present study, the results of real-time PCR showed that the expression of mRNA for RhoA was higher in the urothelium than in the smooth muscle of the pig bladder. The amount of activated RhoA was also greater in the urothelium than in the smooth muscle. These findings suggested that RhoA was more abundant and showed higher activity in the urothelium than in the smooth muscle of the pig bladder. Our *in vitro* functional study suggested that Y-27632, a ROCK inhibitor, could either directly or indirectly inhibits carbachol-induced contraction in the pig urinary bladder. For bladder muscle tissue strips without mucosa, the maximum decrease of tension after addition of Y-27632 (1 nM-100 μM) from the baseline tension obtained by pre-contraction with KCl and carbachol was only by 28.0% and by30.6%, respectively, which was not significantly different from the control value (vehicle). For muscle strips without mucosa, Emax on CRC to carbachol did not decrease significantly after administration of 3 μM Y-27632, but it was decreased significantly by 10-30 μM Y-27632. Considerable relaxation of the contractile response to electrical field stimulation was only noted at a high concentration of Y-27632 (30 μM). From the above results, the inhibitory effect of Y-27632 on pig bladder contraction appeared weaker than in previous studies of rats and human bladders. In tissues with intact mucosa, the maximum decrease of tension after addition of Y-27632 (1 nM-100 μM) from the baseline tension obtained by pre-contraction with KCl and carbachol was by 52.0% and by 53.1%, respectively, which was significantly greater than in the tissue strips without mucosa. With intact mucosa, the inhibitory effect of Y-27632 (3-30 μM) shown by carbachol CRC was significant and concentration-dependent. A significant, concentration-dependent inhibitory effect of Y-27632 was also demonstrated on the increase of tension in response to electrical field stimulation (at 20 Hz and 50 Hz) in tissue strips with mucosa, and inhibition was stronger than in tissues without mucosa.

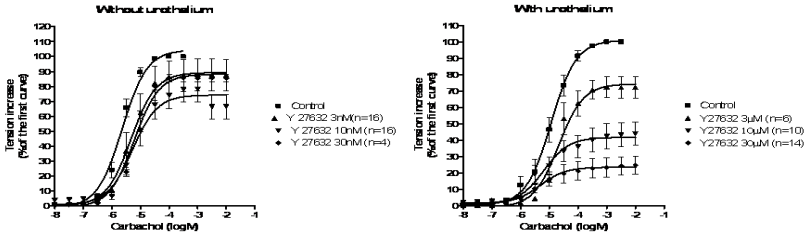
Concluding message
The expression of RhoA mRNA and RhoA activity were greater in the urothelium than in bladder smooth muscle. Y-27632 showed a stronger inhibitory effect on tissue preparations with intact urothelium than on those without urothelium. The present study indicated that Y-27632 has certain inhibitory effects on the contraction of porcine bladder, and this effect may be enhanced by the presence of urothelium.

References

1. Rho-kinase and effects of Rho-kinase inhibition on the lower urinary tract. *Neurourol Urodyn* **26:**948-954,2007.

Figure

Effect of Y-27632 (3, 10, and 30 μM) on concentration-response curve to carbachol in pig bladder. left): bladder tissue strips without urothelium right): bladder tissue strips with urothelium.



Specify source of funding or grant	**This work was supported by a Grant-in-Aid for Scientific Research (18591770) from the Ministry of Education, Science, Sports, Culture and Technology of Japan.**
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?	**No**
Statement that no ethical approval was needed	**Considering that the tissues were obtained from animals killed at a local abattoir, the protocol was thought to be ethical.**