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RHO/ RHO KINASE IS PREDOMINANTLY PRESENT IN THE UROTHELIUM CONTROLLING THE CONTRACTION OF THE HUMAN URINARY BLADDER IN VITRO

Hypothesis / aims of study

The Rho/Rho kinase pathway has been reported to be important in regulating cell morphology and migration by reorganizing the actin cytoskeleton. Recently, Y 27632, a Rho kinase inhibitor, has been shown to relax vascular and nonvascular smooth muscles. The present study investigates whether, or not, the expression of Rho is present in the urothelium or smooth muscle of the human bladder, and the effects of Y 27632 on carbachol-induced contraction in tissues of the human urinary bladder with or without urothelium in vitro.

Study design, materials and methods

Samples of detrusor muscle from the dome region of the human urinary bladder (without cancer) were obtained from 31 patients (male 25, female 6; aged 61- 93 years, mean age 67.7 years) undergoing total cystectomy for bladder cancer or suprapubic prostatectomy for benign prostatic hyperplasia. The study has been conducted in accord with the Helsinki Declaration. The procedures have been approved by the local ethics committee, and written informed consent was obtained from each patient before entry into the study. The mRNA and protein levels of RhoA were analyzed usung the relative multiplex RT-PCR and Western blotting (n=8). Immunohistochemistry, using the same specific antibodies as antibodies for Western blotting, was performed to support the data obtained by Western blotting. The expression levels of these genes were compared between urothelium and smooth muscle of the bladder. In functional studies in vitro, strips of tissue (8mm x 2mm) were cut from the bladder dome, with mucosa removed in 11 tissues and mucosa intact in 12. The tissues were mounted in 10ml organ baths containing Krebs solution which was maintained at 37^{o}C and continuously gassed with 95% O_2 and 5%CO_2. The tissues were subjected to a resting tension of 1g and allowed to equilibrate for 60 minutes. Cumulative concentration-response curves (CRCs) to carbachol were obtained. Tissues were then washed for about 45 minutes until a steady resting level of tension was attained, and were then equilibrated for 30 min with Krebs solution containing the 10μM of Y 27632 or vehicle (time control). After incubation for 30 minutes, a second CRC to carbachol was constructed in the continued presence of Y 27632 or vehicle.

Results

On the relative multiplex RT-PCR, RhoA and Rho kinase were more abundant in the urothelium than in the smooth muscle ($p=0.0031$ and $p=0.0045$, respectively). Western blotting also demonstrated significantly greater expression of RhoA and Rho kinase protein expression in the urothelium than in the smooth muscle ($p<0.0001$ and $p<0.0001$, respectively). Urothelial cells showed moderate to strong reaction for RhoA and Rho kinase antibodies, and smooth muscle cells showed weak to moderate reaction on immunohistochemistrical study.

On functional studies in vitro, Maximal response (Emax) in tissues without mucosa was 9.74$\pm$1.38 gram and that with mucosa was 7.18$\pm$1.26. Maximal response relative to 80mM potassium chloride (%Emax) in tissues without mucosa was 134.9$\pm$9.5% and that with intact mucosa was 94.2$\pm$4.0%. Although the difference in Emax between two tissues were insignificant ($p=0.1876$), %Emax in tissues without mucosa was significantly greater than that with intact mucosa ($p=0.0010$). pEC_{50} (negative logarithm of the molar concentration of carbachol resulting in 50% of the maximum response) in tissues with and without mucosa was 6.0$\pm$0.2 and 6.5$\pm$ 0.2, respectively, the difference being significant ($p=0.0180$). In tissues where mucosa was removed, %Emax after administration of 10μM Y 27362 (126$\pm$9.8%) did not change significantly ($p=0.0606$), compared with those of the first curve. However pEC_{50} decreased significantly ($p=0.0011$) after administration of Y 27362 (6.0$\pm$0.2).

In tissues with intact mucosa, %Emax and pEC_{50} after Y 27362 (10μM) administration were 63.6±6.6% and 5.3±0.2, respectively. %Emax and pEC_{50} decreased significantly (p=0.0007, P= and p=0.0046) after administration of Y27632.

Interpretation of results

From the results of the Relative multiplex RT-PCR, Western Blotting and Immunohistochemistry, the expressions of Rho and Rho kinase were predominantly found in the urothelium. The maximum responses of bladder smooth muscle without urothelium were significantly greater than those with intact urothelium, suggesting the presence of a diffusible, urothelium-derived inhibitory factor. Y27632 significantly decreased %Emax in tissues with intact mucosa, but not in those without mucosa.Thus the inhibition of carbachol-induced contraction by Rho kinase inhibitor may be noted in the presence of urothelium.

Concluding message

Rho and Rho kinase appear to be predominantly present in the urothelium. The maximum response to carbachol was decreased in the presence of urothelium. The Rho/Rho kinase pathway appears to be involved in the contraction of human bladder smooth muscle in the presence of urothelium.