

INHIBITION OF RHO-KINASE REDUCES SPONTANEOUS MYOGENIC TONE IN THE FEMALE PIG ISOLATED URETHRA

Aims of Study

Smooth muscle contraction in response to some agonists acting on G-protein-coupled receptors, involves the GTPase, Rho, and one of its effectors, Rho-associated kinase. The Rho-Rho-kinase pathway modulates the level of phosphorylation of the myosin light chain of myosin II, mainly through inhibition of myosin phosphatase, and contributes to agonist-induced Ca^{2+} sensitization in smooth muscle contraction. The compound, Y27632, has been reported to specifically inhibit the Rho-kinase, and hereby to attenuate agonist-induced contraction of vascular and non-vascular smooth muscle tissues (1). Thus, the Rho-kinase pathway appears to have important functions in agonist-induced Ca^{2+} -sensitization in smooth muscle, but its role in the development of myogenic tone is not well characterised. The aim of the present study was to investigate the effects of Y27632 in the female pig urethra, a preparation, which spontaneously develops tone (2).

Methods

Bladder and urethra from young (6 months) female pigs were removed in a slaughterhouse, shortly after the animals had been killed. The tissue was kept in cold Krebs solution and used within 1 hour. Urethral strips were dissected transversally 3-4 cm distal from the urethro-vesical junction, and were then suspended in thermostatically controlled organ baths (5ml., 37°C) containing Krebs solution, and bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). Isometric tension was recorded by means of a Grass Instruments FT03C force-transducer connected to a Grass 7D polygraph (Grass Instruments Co, MA, USA).

During an equilibration period of 40 min., tension was adjusted until mean stable tension levels were obtained. The preparations were then exposed to Ca^{2+} -free Krebs solution to establish the base-line level. After addition of Ca^{2+} containing Krebs solution, a stable tone was re-established. Concentration-response curves for the Rho-kinase inhibitor Y27632 ($0.1 \mu\text{M}$ - $10 \mu\text{M}$) were then determined. The degree of inhibition was expressed as a percentage of maximum tone. Student's paired two-tailed t-test was used for statistical comparison between before and after giving a drug. A probability of $p < 0.05$ was accepted as significant. All statistical calculations are based on the number of individuals. The values for the negative logarithm of the drug concentration producing half-maximal relaxation ($-\log\text{IC}_{50}$) were determined graphically for each curve by linear interpolation. This project was supported by the Swedish Medical Research Council, grant no. 6837, and the Medical Faculty of Lund University, Sweden.

Results

All preparations developed spontaneous tone, reaching a mean maximum of 6.8 ± 0.9 mN ($n=36$, $P < 0.05$) from base-line level. At the lowest investigated concentration of Y27632 ($0.1 \mu\text{M}$), no relaxant activity was observed. However, above this concentration ($0.3 - 10 \mu\text{M}$), the drug produced concentration-dependent relaxations (Figure 1).

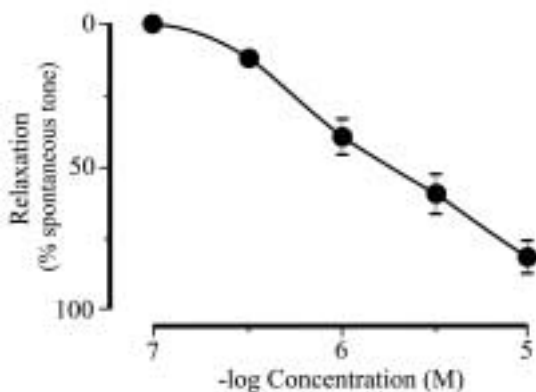


Figure 1. Effect of Y27632 on spontaneous tone in the female pig isolated urethra. Values are given as mean \pm SEM.

The relaxant effect of Y27632 developed slowly and after addition of each concentration of the drug, 4 to 6 minutes were required for a new stable level of tension to develop (Figure 2).

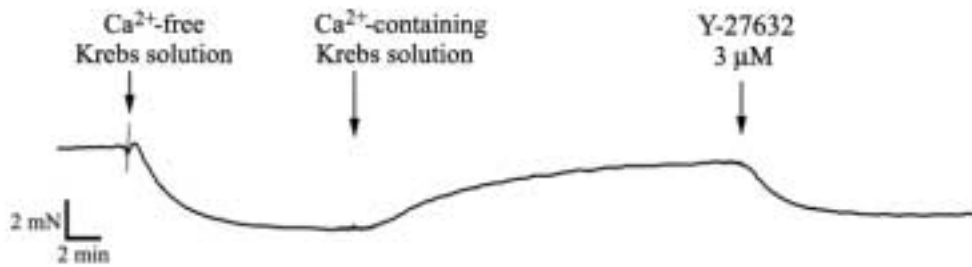


Figure 2. Tracing describing the relaxant effect of 3 μM Y27632 in one spontaneously active urethral preparation from the female pig.

The largest mean relaxant response amounted to $81 \pm 5\%$ ($n = 6$) was obtained at the highest investigated concentration of Y27632 (10^{-6} M). The $-\log IC_{50}$ for Y27632 in spontaneously active urethral preparations was 5.82 ± 0.11 .

Conclusions

The Rho-kinase inhibitor, Y27632, produced concentration-dependent relaxations of spontaneously contracted urethral preparations from the female pig. These results clearly indicate that the Rho / Rho-kinase pathway is involved in the mechanisms resulting in spontaneous myogenic tone of the pig urethra.

References

1. Rho-Rho-kinase pathway in smooth muscle contraction and cytoskeletal reorganization of non-muscle cells. *Trends Pharmacol. Sci.* 22 (1), 32- 39, 2001
2. Factors involved in the relaxation of female pig urethra evoked by electrical field stimulation. *Br. J. Pharmacol.*, 116, 1599-1604, 1995.