

THE EFFECT OF L-NG-NITROARGININE METHYL ESTER (L-NAME) ON RELAXATION INDUCED BY Y 27632, A RHO-KINASE INHIBITOR, IN THE PORCINE URINARY BLADDER WITH INTACT UROTHELIUM

Hypothesis / aims of study

Previously, we reported that the expression of RhoA mRNA and activated RhoA protein were greater in urothelium than in bladder smooth muscle, and that Y-27632, a Rho-kinase inhibitor, showed a stronger inhibitory effect on tissue preparations with intact urothelium than on those without urothelium [1]. ROCK has been reported to downregulate endothelial nitric oxide synthase (eNOS)[2]. Thus we speculated that the relaxation induced by Y 27632 in the intact urothelium could be related to NO in the urothelium. The aims of the present study is to investigate the effect of L-NG-Nitroarginine Methyl Ester (L-NAME), a NOS inhibitor, on relaxation induced by Y 27632 for concentration-response curves to carbachol in the porcine urinary bladder with intact urothelium.

Study design, materials and methods

Urinary bladders from female pigs were collected from an abattoir. Strips of tissue were cut from the bladder dome, and the serosa was removed. Tissues were immediately placed in Krebs solution (in mM: NaCl 118.4, KCl 4.7, CaCl₂ 1.9, NaHCO₃ 24.9, MgSO₄ 1.15, KH₂PO₄ 1.15, and glucose 11.7). A strip of tissue (8 X 2 mm) were cut from the bladder dome and were mounted in 5-ml organ baths containing Krebs solution, and was aerated continuously with 95% O₂ and 5% CO₂. The strips were placed under a resting tension of 1g and then were allowed to equilibrate for 60 min, during which time they were washed every 10 min and the resting tension was adjusted. The isometric tension generated by the tissue was measured by a Power Lab data acquisition system (Analog Digital Instruments, Sydney, New South Wales, Australia). Increasing concentrations of carbachol were added cumulatively in 0.5 log unit increments and concentration-response curves (CRCs) were obtained. Then the tissue specimens were washed for about 60 min until a steady resting tension of 1g was attained, after which equilibration was done for 30 min with Krebs solution containing 30 μ M Y-27632 or vehicle. After incubation for 30 minutes, a second CRC to carbachol was determined in the presence of Y-27632 or vehicle. Again the tissue specimens were washed for about 60 min until a steady resting tension of 1g was attained, after which equilibration was done for 30 min with Krebs solution containing 30 μ M L-NAME plus 30 μ M Y-27632 or vehicle. After incubation for 30 minutes, a third CRC to carbachol was determined in the presence of 30 μ M L-NAME plus Y-27632 or vehicle.

Agonist potencies and the maximum responses were expressed as the mean pEC₅₀ (negative logarithmic value of the molar concentration of carbachol producing 50% of the maximum response) and the mean maximum contraction (E_{max}), respectively. Data were normalized to the maximum response generated by the first curve, and percentage of the maximal response was expressed as the mean \pm SEM.

Results

The maximum response to carbachol (E_{max}) of muscle strips with mucosa (control) was 4.14 \pm 0.14g (n=16), and that in the presence of 30 μ M Y-27632 and in the presence of 30 μ M L-NAME plus 30 μ M Y-27632 was 2.24 \pm 0.39 (54.8 \pm 10.9% of control) and 1.44 \pm 0.34 (34.4 \pm 8.4% of control), respectively. There was a significant difference with regard to E_{max} value between the control and that in the presence of 30 μ M Y-27632 and in the presence of 30 μ M L-NAME plus 30 μ M Y-27632 (p<0.0001). However no significant difference was noted between E_{max} in the presence of 30 μ M Y-27632 and that in the presence of 30 μ M L-NAME plus 30 μ M Y-27632. The pEC₅₀ in the control, and that in the presence of 30 μ M Y-27632 and in the presence of 30 μ M L-NAME plus 30 μ M Y-27632 were 4.42 \pm 0.16, 5.42 \pm 0.22 and 5.04 \pm 0.23. There was a significant difference with regard to EC₅₀ value between the control and that in the presence of 30 μ M Y-27632 and in the presence of 30 μ M L-NAME plus 30 μ M Y-27632 (p<0.05), but no significant difference between the latter two values (Fig).

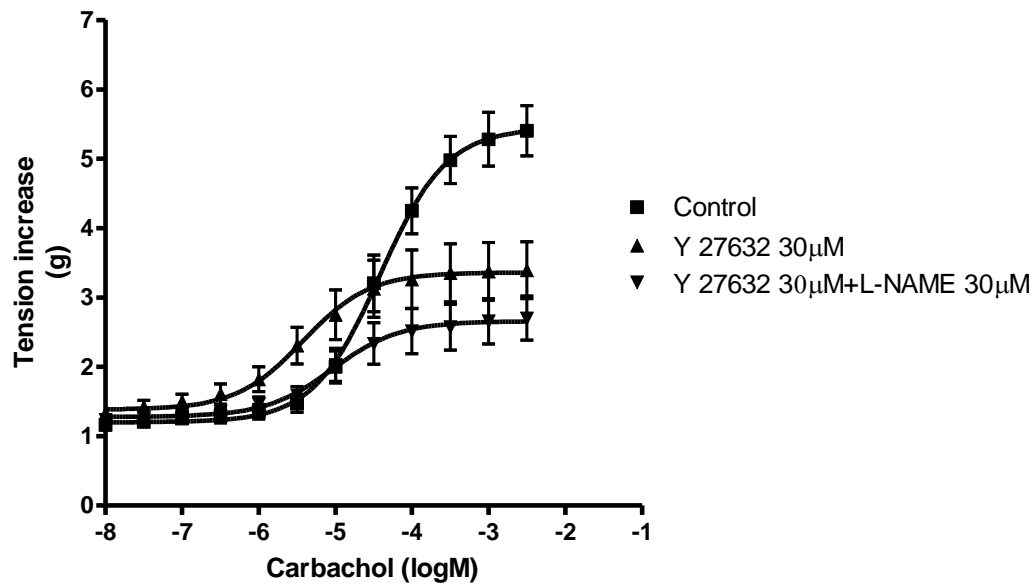
Interpretation of results

The maximum response and EC₅₀ on CRC to carbachol was decreased by 45% in the presence of Y-27632 with intact urothelium. However, NOS inhibitor had no effect on the inhibitory effect of Y-27632.

Concluding message

The relaxation induced by Rho-inhibitor did not seem to relate to nitric oxide in the presence urothelium.

**The effect of Y 27632 and L-NAME,
on concentration-response curve to carbachol
in the porcine urinary bladder with intact urothelium**



References

1. Neurourol Urodynam 28:521-528, 2009.
2. Circulation 106:57-62, 2002..

Disclosures

Funding: None **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Pig **Ethics not Req'd:** Pig bladder was obtained from the dead animal