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INVOLVEMENT OF RHO-KINASE IN THE REGULATION OF SPONTANEOUS TONE IN THE FEMALE PIG URETHRA

Aims of Study

Contraction of smooth muscle is primarily regulated by reversible phosphorylation of Ser-19 on the regulatory light chain on myosin. The phosphorylation of RLC is catalysed by myosin light chain kinase (MLCK) and the dephosphorylation by a type 1 phosphatase, myosin light chain phosphatase (MLCP). Thus the amount of force generated is determined by the phosphorylation/dephosphorylation rate ratio The activity of MLCK is mainly regulated by calcium/calmodulin, but other signalling pathways have been shown to modulate the activity of both MLCK and MLCP at constant calcium concentration [1]. Calcium desensitisation is the term used to describe the increase in force at constant calcium by contractile agonists. This is brought by agonist activation of G-protein coupled receptors, which cause an exchange of GTP for GDP on the GTPase RhoA. This complex activates Rho-kinase that phosphorylates the myosin binding subunit of MLCP, which diminishes its phosphatase activity leading to an increase in RLC phosphorylation and thereby force [1]. The circular smooth muscle of pig urethra has spontaneous tone that is resistant to neural blockade and muscarinic antagonists. This suggests that the tone is generated within the smooth muscle cells themselves [2]. In the present study we set out a set of experiments to test if the Rho family GTP-ases and one of their downstream mediators, Rho-kinase, are regulators of spontaneous tone in the pig urethra.

Methods

Circular smooth muscle bundles from pig urethra were dissected out and mounted for registration of isometric force in small organ baths. The effect of Y-27632 on development of tone was investigated after an initial contraction-relaxation cycle. The relaxant effects of felodipine and Y-27632 on tone were also studied. The role of Rho-GTPases, Rho, Rac, and Cdc42, were examined using the glucosyltransferase toxin B from clostridium difficille (Toxin B), which was delivered intracellulary by overnight incubation in a solution containing 40 ng Toxin B/ml. For measurement of intracellular calcium muscle bundles were mounted on glass capillaries and loaded with 16μmol/L fura-2 AM in Krebs solution in the dark for 3 hours. A indirect estimation of global intracellular calcium was obtained be recording the epifluorescence from 340 and 380 nm excitation of fura-2 with an emission wavelength of 510 nm using an IonOptix imaging system [3].

Results

The muscle preparations slowly developed spontaneous tone in calcium containing Krebs solution. Removal of calcium caused a slow relaxation towards the passive force level, and when calcium was re-added force recovered to the initial level. Incubation overnight in Toxin B inhibited the development of spontaneous force whereas the force of bundles incubated without toxin was similar to that of fresh preparations. On the other hand, the force development after activation using high-K^+ (120 mM) or 100 μM noradrenaline was similar in toxin B treated preparations and controls. Y-27632 reversible inhibited the development of spontaneous force and when the drug was added on the force plateau it caused a concentration dependent relaxation. The maximal relaxation was 81 ± 13 % and the apparent K_i was 2.3 ± 1.1 μM. On addition of calcium to the organ bath, the 340/380 ratio increased before force development. The rate of increase in 340/380 ratio was faster than the corresponding increase in force. Addition of 10 μM Y-27632 had no effect on the 340/380 ratio, whereas 10 μM felodipine caused an instant decrease in ratio that decreased towards the level in calcium free solution.

Conclusions

The data presented directly demonstrate and confirm earlier reports that influx of calcium is obligatory for the development of spontaneous tone in the pig urethra. They further suggest that the Rho GTP-ases Rho, Rac or Cdc42 are involved in the generation of spontaneous

tone. The apparent Ki for the inhibition of force by Y-27632 is similar to that reported for the *in vitro* inhibition of Rho-associated kinase. This suggests that Rho-associated kinase is one the downstream mediators of a Rho-GTPase.

References

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