583

Malmqvist U[1], Hedlund P[1], Swärd K[2], Andersson K[1]
1. Department of Clinical Pharmacology, 2. Department of Physiological Science

MUSCARINIC RECEPTOR SIGNALLING IN PIG AND RAT BLADDER.

Aims of study

The major signal to activate the contractile system in smooth muscle is an increase in the intracellular calcium concentration. In the cell calcium binds to calmodulin and this complex activates myosin light chain kinase (MLCK) that reversible phosphorylates Ser-19 on the regulatory light chain on myosin. The dephosphorylation is catalysed by a type 1 phosphatase, myosin light chain phosphatase (MLCP). The amount of force generated is determined by the phosphorylation/dephosphorylation activity ratio. In addition to calcium, other signalling pathways can modulate smooth muscle contraction [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 causes 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 phospatase activity leading to an increase in RLC phosphorylation and force [1]. In the present study we characterised some of the signalling pathways involved in the muscarinic excitation-contraction coupling in urinary bladders from rat and pig.

Methods

Smooth muscle bundles from female pig and rat bladder were dissected out and freed from fat and epithelium and mounted for registration of isometric force in small organ baths. The preparations were initially activated using high-K^+ (120 mM) and when stable contractions were established the effects of agonists or antagonists were investigated. All force values reported are normalised to the mean force of the two last high-K^+ induced contractions prior to the start of the experimental protocol. In experiments using activation with high-K^+ the effects of release of excitable transmitters from depolarised nerves were blocked using a cocktail containing indomehacin, propranolol, phentholamine and scopolamine. The glucosyltransferase toxin B from clostridium difficille (Toxin B) which inactivates Rho, Rac and Cdc42 was delivered intracellulary by overnight incubation in a solution containing 40 ng Toxin B/ml. Intracellular calcium was determined using the Fura-2 technique. Muscle bundles were attached to 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 by recording of the epifluorescence from 340 and 380 nm excitation at an emission wavelength of 510 nm using an IonOptix imaging system [2].

Results

When preparations from pig and rat urinary bladder were activated using 120 mm KCl, Y-27632 concentration dependently inhibited force. The maximal inhibition of force was larger in pig bladders compared to rat bladders, 93±2 % and 44±10 % for pig and rat bladders respectively. The sensitivity to carbachol was similar for rat and pig bladder with maximal force at 10 μM and an EC50 value of about 150 nmol. The maximal force was 120 % and 170 % of the response to 120 mM KCl for rat and pig bladder respectively. Carbachol induced a biphasic force response, an initial rapid increase to peak force followed by a decline towards a stable force plateau. The calcium signal showed the same biphasic behaviour as the force response. Y-27632 inhibited carbachol induced contraction in both rat and pig bladders with an apparent inhibition constant of about 1 μM in pig and rat bladders. Y-27632 had no effect on the calcium transient. Incubation overnight in Toxin B inhibited the initial peak force whereas the plateau phase was completely abolished. The force response of bundles incubated without Toxin B was similar to that of fresh preparations.

Conclusions

The inhibitory effect of Y-27632 on KCl induced contraction suggests that calcium sensitisation mechanisms are activated under basal conditions or that Y-27632 has unspecific effects. The data presented suggests that the Rho-GTP-ases Rho, Rac or Cdc42 are involved in the regulation of urinary bladder smooth muscle contraction after muscarinic activation. The

data indicate that the plateau force is dependent on calcium sensitisation mechanisms despite a high calcium concentration at this phase of the contraction.

References

1. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. J. Physiol. 522, 177-185 (2000)
2. Long-term regulation of contractility and calcium current in smooth muscle. Am. J. Physiol. 273, C1714-C1720 (1997).