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CARBACHOL INDUCED CALCIUM SENSITIZATION IN RAT URINARY BLADDER

Hypothesis / aims of study

Smooth muscle contraction is normally thought to be activated by an increase in cytosolic calcium produced by calcium entry through voltage sensitive calcium channels or by calcium release from the sarcoplasmic reticulum. Agonists activating G protein coupled receptors increase force in smooth mucle via myosin light chain phosphorylation. The ratio of activities of calcium/calmodulin dependent myosin light chain kinase and myosin light chain phosphatase determines the level of myosin light chain phosphorylation and the activation of the muscle. There are, however, mechanisms which cause agonist induced calcium sensitization that can lead to muscle contraction without any necessary change in intracellular calcium. These mechanisms, which operate through pathways involving Rho kinase and protein kinase C, are thought to inhibit myosin phosphatase activity at constant calcium, and therefore cause an increase in force (1). The present study was designed to investigate carbachol induced calcium sensitization in β-escin-permeabilized rat bladder, and to determine the signal transduction pathways involved, by using kinase inhibitors and inhibitors of intracellular calcium signalling pathways.

Study design, materials and methods

Small strips were dissected from the detrusor of bladders from Wistar rats. These were mounted in a fixed position in Hepes buffered modified Krebs' solution at room temperature under a resting tension of 100 mg in one of a series of small chambers in a Perspex strip. Solution changes were made by moving the Perspex strip. After stable responses had been achieved to 80 mM K^+ and 50 μM carbachol in intact strips, they were moved into relaxing solutions, incubated for a few minutes and then permeabilized with 40 μM β-escin in relaxing solution at pH 6.8. Relaxing solution contained (mM) K propionate 130; $MgCl_2$ 4; Na_2ATP 4; tris-maleate 20; creatine phosphate 10; EGTA 4 and creatine phosphokinase 3.3 units/ml. Activating solution was the same as relaxing solution except that EGTA was lowered to 0.05 mM and calcium was added. Free calcium concentration was changed by adding an appropriate amount of $CaCl_2$ to activating solution and the negative logarithm of the free calcium concentration (pCa) was calculated using a computer programme. Protease inhibitor leupeptin (1 μM) and mitochondrial blocker FCCP (1 μM) were also added to the activating solution. The contractile force was measured by a sensitive force transducer (ADInstruments) connected to an Apple Macintosh PowerBook 1400c computer using Chart software and MacLab 8 hardware.

Results

Calmodulin (1 μM) and GTP (100 μM) caused significant contractions when applied to β-escin permeabilized rat bladder strips when the calcium concentration was clamped at pCa 6. Under these constant calcium conditions carbachol (50 μM) added to the bath caused a further contraction. These carbachol-induced contractions were significantly inhibited by the muscarinic receptor antagonist atropine (50 μM). The contractions were also inhibited by a Rho kinase inhibitor, Y-27632 (5 μM), and by a protein kinase C inhibitor GF 109203X (5 μM). All these responses occurred in the presence of sarcoplasmic reticulum Ca^{+2}ATPase inhibitor CPA (1 μM). 2-APB (30 μM) reduced the carbachol induced contraction whereas ryanodine (5 μM) had no effect.

Interpretation of results

β-escin permeabilization preserves the receptor-effector pathways in smooth muscles. In rat bladder smooth muscle calmodulin and GTP are lost during skinning, but if these are included in the bathing solution, carbachol, through activation of muscarinic receptors can induce contraction at constant calcium concentration-(pCa 6). In other words, muscarinic receptor activation can induce significant calcium sensitization in this tissue. Our results show that this calcium sensitization in rat bladder smooth muscle involves both rho kinase and protein kinase C pathways. These pathways are known to independently control the activity of

myosin light chain phosphatase (1). This sensitization is independent of calcium release from stores since it occurs when the calcium uptake into the stores has been inhibited by CPA, and also when calcium-activated calcium release from the stores is inhibited by ryanodine. The inhibitory effect of 2-APB under our conditions is likely to be caused by inhibition of IP_3 receptors since the significant effect of 2-APB was only observed in the absence of CPA.

Concluding message

Muscarinic receptor activation is normally thought to induce contraction in the bladder through IP_3 production and release of calcium form internal stores (2). We have, however shown that muscarinic receptor activation can induce calcium sensitization in rat detrusor smooth muscle, and it is likely that this plays a significant role in activation of bladder contraction under normal conditions. Modulation of these pathways might represent a target for drug development to treat the over-active bladder.

References

(1) Ca^{2+} Sensitivity of Smooth Muscle and Nonmuscle Myosin II: Modulated by G Proteins, Kinases, and Myosin Phosphatase (2003) Physiol. Rev., **83**: 1325-1358.

(2) Agonist-induced contraction and accumulation of inositol phosphates in the guinea-pig detrusor: evidence that muscarinic and purinergic receptors raise intracellular calcium by different mechanisms (1990) J. Urol., **144**: 775-779.