297

Durlu-Kandilci N T [1], Brading A F[2]
1. Hacettepe University, 2. Oxford University

A COMPARISON OF THE EFFECTS OF RHO KINASE AND PROTEIN KINASE C ON CALCIUM SENSITIZATION IN RAT AND GUINEA PIG BLADDERS

Hypothesis / aims of study

Smooth muscle muscarinic receptor activation increases cytoplasmic free calcium either through entry from voltage dependent channels or release from the sarcoplasmic reticulum (1). When calcium binds to calmodulin, myosin light chain kinase (MLCK) is activated leading to muscle contraction. This reaction is reversed by myosin light chain phosphatase (MLCP). In smooth muscle, there are also other mechanisms that induce contraction. Under constant intracellular calcium concentration and MLCK activity, agonists activating G-protein coupled receptors may cause contraction by inhibiting MLCP activity, a situation named as *calcium sensitization*. MLCP inhibition involves two pathways whose determinants are Rho kinase and protein kinase C (PKC) (2). In the present study, the intracellular mechanisms of calcium sensitization were compared in β-escin skinned rat and guinea pig bladders.

Study design, materials and methods

Small strips were dissected from the detrusor of bladders from Wistar rats and guinea pigs. 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 block. Solution changes were made by moving the Perspex block. 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 / 80 μM β-escin for rat / guinea pig 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; 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 its negative logarithm (pCa) was calculated using a computer programme. Sarcoplasmic reticulum Ca^{+2}ATPase inhibitor CPA (1 μM), 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. Data were expressed as a percentage of 80 mM K^+ in intact strips.

Results

When the calcium concentration was clamped at pCa 6, calmodulin (1 μM) and GTP (100 μM) caused significant contractions in β-escin permeabilized rat and guinea pig bladder strips. Under these constant calcium conditions, carbachol (50 μM) added to the bath caused a further contraction (sensitization) in both bladders. The contraction responses were inhibited by muscarinic receptor antagonist atropine (50 μM) in both tissues with different ratios; 47.94 ± 1.71 % in rat and 35.33 ± 3.98 % in guinea pig (n=4-5). These carbachol induced contractions were significantly inhibited by a Rho kinase inhibitor, Y-27632 (5 μM) in rat (55.20 ± 8.42 %) but not in guinea pig bladder. However, a PKC inhibitor GF 109203X (5 μM) inhibited carbachol induced sensitization in both tissues; 38.44 ± 5.76 % in rat and 34.06 ± 3.21 % in guinea pig (n=4).

Interpretation of results

The receptor-effector coupling is preserved during β-escin permeabilization in smooth muscle. Calmodulin and GTP smaller than the holes opened by β-escin in the plasma membrane were lost during skinning. When these were replaced, calmodulin interacting with calcium activated MLCK and then GTP activated G proteins, both inducing contractions. In the presence of these exogenous agents and at constant calcium, muscarinic receptor agonist carbachol induced a further contraction (calcium sensitization) in both rat and guinea pig. This

sensitization was independent of calcium release from stores since it occurs when the calcium uptake had been inhibited by CPA. The muscarinic receptor activation was confirmed by atropine inhibition. However, the calcium sensitization pathways differed between these two species. In rat bladder, calcium sensitization was induced by both rho kinase and PKC pathways, former being the major one. On the other hand, in guinea pig bladder, calcium sensitization only involved PKC pathway.

Concluding message

In detrusor, muscarinic receptor activation generally causes contraction by IP_3 production and the release of calcium from internal stores (3). In the present study, we emphasized that muscarinic receptor activation can induce calcium sensitization in rat and guinea pig detrusors. We have also shown that there are species differences in calcium sensitization control mechanisms. Since it is likely that sensitization plays a significant role in activation of bladder contraction under normal conditions, luminating these pathways might probably be useful for new drug developments against over-active bladders.

References

1. Physiol. Rev., **76**, 967-1003 (1996).
2. J. Physiol., **522**, 177-185 (2000).
3. J. Urol., **144**, 775-779 (1990).