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THE SPECIFIC TYPE 4 PHOSPHODIESTERASE INHIBITOR ROLIPRAM ACTIVATES LARGE AND INTERMEDIATE CONDUCTANCE CA2+-ACTIVATED K+ CHANNELS IN PIG BLADDER NECK: A POSSIBLE THERAPEUTICAL TARGET FOR TYPE III STRESS URINARY INCONTINENCE.

Hypothesis / aims of study

Relaxation of bladder and urethra is produced, in part, via cAMP (PKA)- or cGMP (PKG)-dependent protein kinase pathways, respectively [1]. Phosphodiesterase type 4 (PDE4) and 5 (PDE5) inhibitors may be useful for the treatment of urge incontinence and low compliance bladder [2]. The knowledge of the mechanisms involved in the control of the bladder neck smooth muscle tone, structure that together with the urethra comprise the outflow bladder region, is essential for the treatment of type III stress urinary incontinence due to intrinsic sphincteric deficiency [3]. Previous studies, carried out in our laboratory, have shown that rolipram, a selective PDE4 inhibitor, is the phosphodiesterases inhibitor that produced a more potent relaxation of bladder neck. There are no data, however, about the signalling pathways underlying in the rolipram-induced relaxation. Therefore, the current study investigates the mechanisms involved in the rolipram-induced relaxation in the pig bladder neck.

Study design, materials and methods

Urothelium denuded strips 4-6 mm long and 2-3 mm wide were suspended horizontally with one end connected to an isometric force transducer (Grass FT 03C) and the other one to a micrometer screw, in 5 ml organ baths containing physiological saline solution (PSS) at 37^{o} C gassed with carbogen (95% O_2 and 5% CO_2) to obtain a final pH of 7.4. The signal was continuously recorded on a polygraph (Graphtec Multicorder MC 6621). Passive tension of 2 g was applied to the strips and they were allowed to equilibrate for 60 min. On 1 μM phenylephrine (PhE)-induced tone, cumulative concentration-response relaxation curves (CRC) to rolipram were obtained by increasing the organ bath concentration in half log unit steps. Since CRC to this drug was not reproducible in two consecutive curves, bladder neck strips from the same animal were run in parallel, one of them used as control and the other one assessed for the specific treatment for 30 min.

Results

Rolipram produced concentration-dependent relaxations of the pig bladder neck. A threshold concentration (30 nM) of the adenylyl cyclase activator forskolin evoked a leftward displacement of the relaxation CRC induced by rolipram, whereas that KT5720 (3 μM), a selective PKA inhibitor, reduced such effect. In a potassium-enriched physiological saline solution (KPSS, 60 mM), rolipram evoked a lower relaxation in comparison with that obtained on PhE-precontracted strips. Iberiotoxin (100 nM) and TRAM 34 (20 nM), blockers of large (BK_{Ca}) and intermediate (IK_{Ca}) conductance Ca^{2+}-activated K^+ channels, respectively, reduced the relaxation to rolipram. However, blockade of small (SK_{Ca}) conductance Ca^{2+}-activated K^+ channels and ATP-dependent K^+ (K_{ATP}) channels failed to modify such response.

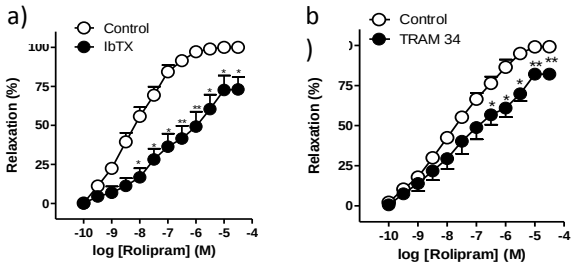

Figure 1 Log concentration-response relaxation curves to rolipram in control conditions (open circles) and in the presence (closed circles) of iberiotoxin (IbTX, 100 nM) (a) and TRAM 34 (20 nM) (b). Results are expressed as a percentage of the phenylephrine-induced contraction and represent mean±SEM of eight preparations. **P*<0.05, paired *t*-test.

Interpretation of results

These results suggest that rolipram produces relaxation of the pig bladder neck through PKA dependent mechanisms involving opening of BK_{Ca} and IK_{Ca} channels.

Concluding message

Blockers of PKA pathway and BK_{Ca} and IK_{Ca} channels may be useful for urinary incontinence treatment produced by intrinsic sphincteric deficiency.

References

1. Andersson and Wein, Pharmacol Rev 56(4): 581-631, 2004.
2. Wheeler et al, J Smooth Muscle Res 41(1):1-21, 2005.
3. Martínez-Sáenz et al, J Urol 186(2): 728-35, 2011.

Disclosures

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