Hypothesis / aims of study

The release of a previously unrecognized smooth muscle relaxant factor by muscarinic receptor stimulation from rat urinary bladder has been demonstrated using a coaxial bioassay system (1-3). The present study was designed to characterize this urinary bladder-derived relaxant factor by the same bioassay consisting of rat bladder as the donor organ and anococcygeus muscle as the assay tissue.

Study design, materials and methods

The rat anococcygeus muscle that was mounted within the bladder was precontracted by phenylephrine (1-3 µM) and then, the concentration-dependent relaxation to cumulatively added acetylcholine (10 nM –1 mM), physostigmine (1, 10, 100 µM) and α,β-methylene ATP (10 nM–0.1 mM) were elicited. Concentration-dependent acetylcholine response was also constructed before and after incubation with the antagonists and inhibitors given below.

Results

Acetylcholine produced concentration-dependent relaxation response in precontracted anococcygeus muscle that was placed within the bladder. This relaxation was not altered by incubation with the antagonists of calcitonin gene-related peptide (CGRP 8-37; 1 µM), vasoactive intestinal peptide (VIP 6-28; 1 µM), NK1 (L-732138; 5 µM), NK2 (MEN-10376; 1 µM), NK3 (SB-218795; 1 µM), purinergic P2 (PPADS; 50 µM) and adenosine (CGS 15943; 1 µM) receptors as well as capsaicin (10 µM) or alpha-chymotrypsin (10 U/ml). The second messangers in the release and/or relaxant effect of urinary bladder-derived relaxant factor were also examined. Adenylate cyclase inhibitor SQ-22536 (100 µM) and protein kinase A inhibitor KT-5720 (1 µM) significantly inhibited the acetylcholine responses while guanylate cyclase inhibitor ODQ (100 µM), and protein kinase C inhibitor H-7 (30 µM) had no effect in the coaxial bioassay system. Acetylcholine esterase inhibitor physostigmine and α,β-methylene ATP, the potent contractile agent of the detrusor muscle produced concentration-dependent relaxation responses in the coaxial-bioassay system similar to that of acetylcholine.

Interpretation of results

These data have shown that the relaxant effect of urinary bladder-derived relaxant factor does not involve the receptors of the peptides of the afferent neurons or the purinergic mediators in the bladder wall. Adenylate cyclase and protein kinase A are involved in the release or relaxant effect of urinary bladder-derived relaxant factor, and this factor could be released in response to endogenous acetylcholine or the contraction of the detrusor muscle.

Concluding message

In conclusion, the urinary bladder-derived relaxant factor that is demonstrated by the coaxial bioassay system is neither one of the peptides present in the afferent neurons of the bladder nor a purinergic mediator. The demonstration of its release by endogenous acetylcholine and in response to contraction of the detrusor muscle suggests its importance in physiological functions of urinary bladder.

References

2. Naunyn-Schmiedebergs Arch Pharmacol (2003) 367(5); 547-552.

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