

SIGNAL TRANSDUCTION UNDERLYING MUSCARINIC RECEPTOR-INDUCED CONTRACTION OF HUMAN BLADDER

Aims of Study

Muscarinic receptors are the physiologically most relevant receptor to elicit bladder contraction. While human bladder co-expresses M₂ and M₃ receptors, contraction occurs predominantly if not exclusively via the M₃ receptor (1,2). Against this background we have investigated the signal transduction mechanisms of human bladder contraction elicited by the muscarinic receptor agonist carbachol.

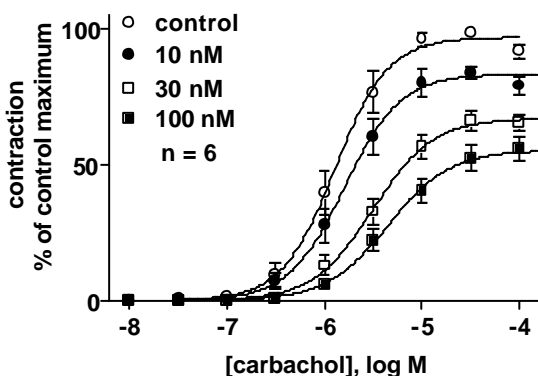
Methods

Bladder tissue was obtained with informed consent from patients undergoing cystectomy due to bladder cancer from cancer free parts of the bladder. Strips (10.4 ± 0.4 mm length, 14.6 ± 1.1 mg weight) were prepared and mounted in organ baths for isometric contraction studies. Four consecutive carbachol concentration-response curves were generated in each strip, first in the absence and then in the presence of the increasing concentrations of inhibitors of the respective signalling pathways. Vehicle time-control strips were studied in parallel in each experiment. Data are expressed as means ± SEM. Statistical significance of differences was assessed by two-way ANOVA testing for main treatment and concentration effect, followed by multiple comparison-corrected post-hoc tests to compare individual inhibitor concentrations to paired time-controls.

Results

In the first concentration-response curve in the absence of inhibitor carbachol had maximal effects of 48.2 ± 3.5 mN and a -log EC₅₀ of 6.08 ± 0.04 mol/l (n = 65). Although M₃ receptors typically couple to phospholipase (PL) C stimulation, the PLC inhibitor U 73,122 (1-10 μM) did not significantly affect the potency or maximal effects of carbachol relative to its vehicle (n = 9 each). Similarly, SK&F 96,365, an inhibitor of store-operated Ca²⁺ channels (1-10 μM), also failed to significantly affect carbachol-induced contraction (n = 9 each). In a smaller number of experiments, the protein kinase C inhibitors Gö 6850 (0.1-1 μM, n = 4) and calphostin (0.1-1 μM, n = 2) also failed to affect carbachol responses. On the other hand, butan-1-ol, an inhibitor of PL D (0.03-0.3%) at least in its highest concentration reduced maximum carbachol responses by approximately 30% relative to its negative control butan-2-ol (n = 8 each, p < 0.001). The most prominent inhibition, however, was seen with the Ca²⁺ entry blocker nifedipine, which inhibited maximum contraction by 20%, 36% and 41% in concentrations of 10, 30 and 100 nM, respectively (p < 0.05, < 0.001 and < 0.001, respectively, n = 6). Neither butan-1-ol nor nifedipine significantly affected the potency of carbachol for its remaining response.

Figure 1: Effects of increasing nifedipine concentrations on the concentration-response curve for carbachol.



Conclusions

Although activation of PL C followed by activation of protein kinase C is the classical signalling response of M_6 receptor stimulation, the present study did not detect a role for this mechanism in human bladder contraction. Store-operated Ca^{2+} channels also appear to be of little relevance. In contrast, PL D and to an even greater extent voltage-operated Ca^{2+} channels appear to play important roles in the excitation-contraction coupling of human bladder. They might be involved in the pathophysiology of bladder dysfunction and/or represent potential therapeutic targets.

References

1. J. Auton. Pharmacol. 21: 243-243, 2001
2. Br. J. Pharmacol. 136: 641-643, 2002