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CYCLIC-AMP-INDEPENDENT RELAXATION INDUCED BY BETA-ADRENERGIC ACTIVATION IN HUMAN DETRUSOR

Abstract Text:

Hypothesis / aims of study

It is well known that the activation of the sympathetic nervous system contributes to urine storage by relaxing the detrusor via activation of beta-adrenoceptors (beta-ARs). Although species difference of the beta-AR subtypes responsible for detrusor relaxation has been reported, the relaxation of human detrusor is mediated mainly via beta₃-ARs.

Until recently, cyclic AMP (cAMP) was considered to be the prototypical second messenger of beta-ARs. However, more recent findings, for instance, in rat detrusor [1] and guinea pig gastrointestinal [2], have questioned its role in mediating smooth muscle relaxation via beta-AR activation. Therefore, we have investigated the intracellular signalling mechanisms underlying beta-AR-mediated relaxation of human detrusor smooth muscle cells.

Study design, materials and methods

The present study was approved by the local Ethics Committee, and written informed consent was obtained from all patients before the operation. Bladder tissue was obtained from the anterior or posterior wall of the bladder body in 8 patients (7 men and 1 woman; 61 to 78 years old, mean age: 70.5 ± 3.0) with bladder carcinoma undergoing radical cystectomy. After the mucosa and adventitia had been removed, detrusor preparations (approximately 10x5x3 mm) were isolated. Each preparation was suspended in a 10-mL organ bath containing Krebs solution; this was maintained at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. One end of each strip was connected to a force-displacement transducer and changes in muscle tension were measured and recorded on a pen-writing oscillograph. The preparation was gradually stretched until a stable tension of 10 mN was obtained. Concentration-response curves for isoproterenol were obtained by cumulative addition of the appropriate concentration to the bathing fluid. Those experiments were performed in the presence and absence of SQ22536 (an adenylate cyclase inhibitor), MDL-12330A (an adenylate cyclase inhibitor), 2',5'-dideoxyadenosine (an adenylate cyclase inhibitor), apamin (a small conductance calcium-activated potassium (SK_{Ca}) channel blocker), 4-aminopyridine (a K_V channel blocker), iberiotoxin (a BK_{Ca} channel blocker), charybdotoxin (a BK_{Ca} channel blocker), or glibenclamide (a K_{ATP} channel blocker). All experiments were conducted in the presence of 1 microM phentolamine, an alpha-adrenoceptor antagonist. The relaxation of muscle was expressed as the percentage of maximal relaxation achieved by 1 mM papaverine at the end of the experiment. Non-linear regression was used to fit sigmoid curves to the isoproterenol concentration-response curves to determine agonist potency (pEC₅₀) and maximum effects (E_{max}). All data are expressed as mean ± SEM of *n* experiments. Statistical significance of inhibitor effects on the pEC₅₀ or E_{max} of isoproterenol was assessed by an unpaired two-tailed t-test, and *p*<0.05 was considered statistically significant.

Results

Effects of adenylate cyclase inhibitors on relaxant responses to isoproterenol.

Isoproterenol relaxed detrusor preparations in a concentration-dependent manner with a pEC₅₀ of 7.09 ± 0.29 and an E_{max} of 86.9 ± 8.8 % (*n*=9). All the three adenylate cyclase inhibitors, SQ22536 (0.1 mM, *n*=5), MDL-12330A (0.3 mM, *n*=4) and 2',5'-dideoxyadenosine (0.3 mM, *n*=3), caused only minor modification of the isoproterenol-induced relaxation without statistically significant inhibition (Fig. 1A).

Effects of potassium (K⁺) channel blocker on relaxant responses to isoproterenol

The SK_{Ca} channel blocker, apamin (1 microM), inhibited the relaxant response to isoproterenol with significant reduction of its pEC₅₀ (apamin(+), 7.00 ± 0.06, *n*=3 vs. control, 7.35 ± 0.12, *n*=4; *p*<0.01) and E_{max} (76.3 ± 4.8 vs. 93.8 ± 4.3; *p*<0.01, Fig. 1B). However, none of the other K⁺-channel blockers, 4-aminopyridine (3 mM, *n*= 2), iberiotoxin (100 nM, *n*=5), charybdotoxin (100 nM, *n*=5), and glibenclamide (10 microM, *n*=4), caused significant effects on the isoproterenol-induced relaxation (data not shown).

Interpretation of results

The main finding of this study is that the beta-adrenoceptor-mediated relaxation in response to isoproterenol in the human detrusor was not diminished when the activity of adenylate cyclase was suppressed by all the three inhibitors used. This indicates that intracellular signalling mechanisms independent of adenylate cyclase/ cAMP pathway are involved in the human detrusor relaxation induced by beta-adrenergic activation. Although we did not focus selectively on beta₃-AR-mediated relaxation in the present study, involvement of such cAMP-

independent mechanisms in β_3 -adrenoceptor-mediated relaxation has been demonstrated in guinea pig gastro-intestinal smooth muscles [2]. Also in the β -AR-mediated relaxation of rat detrusor, a similar cAMP-independent mechanism [1] as well as K_{ATP} channel-mediation has been demonstrated. However, the present results with subtype-selective K^+ -channel inhibitors suggest that the SK_{Ca} channel, but not other K^+ -channels, may partly account for the cAMP-independent mechanism(s) underlying β -AR-mediated human detrusor relaxation. Interestingly, in mice model, detrusor overactivity induced by selective suppression of SK_{Ca} channel has recently been reported [3]. Therefore, activation of SK_{Ca} channel may be a promising therapeutic approach for controlling detrusor overactivity.

Concluding message

We conclude that the relaxation of human detrusor smooth muscles induced by activation of β -ARs is mediated mainly by a cAMP-independent intracellular signalling mechanism, probably including activation of SK_{Ca} channel.

References

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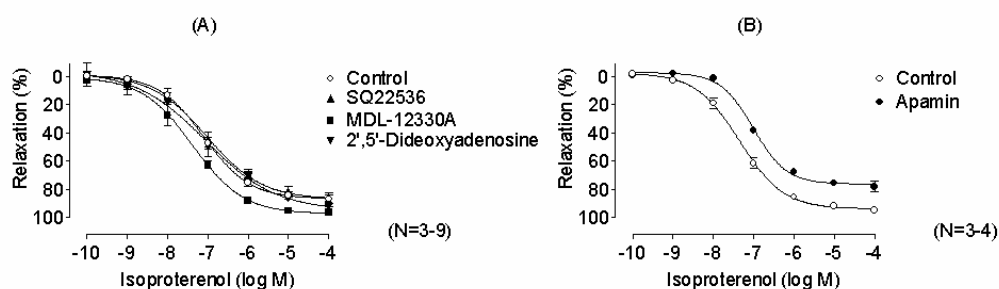


Fig.1 Effects of the adenylyl cyclase inhibitors, SQ22536 (0.1 mM), MDL-12330A (0.3 mM) and 2',5'-dideoxyadenosine (0.3 mM) (A) and the SK_{Ca} channel blocker, apamin (1 μ M) (B) on the isoproterenol-induced relaxation of human detrusor.

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