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UROTHELIUM INHIBITS B-ADRENOCEPTOR-MEDIATED RELAXATION IN HUMAN DETRUSOR SMOOTH MUSCLE VIA CAMP- AND P2 PURINOCEPTOR-DEPENDENT MECHANISM

Hypothesis / aims of study

All three subtypes of β-adrenoceptors (β-ARs), β1-AR , β2-AR , and β3-AR are expressed not only in detrusor smooth muscle cells but also urothelial cells, interstitial cells in the submucosal layer and nerve fibers in the human bladder wall (1). Stimulation of urothelial β-ARs induces releases of nitric oxide (NO) and a factor which inhibits the β-AR agonist-induced relaxation of the human detrusor smooth muscle and that this inhibitory mechanism might not involve NO (2). Moreover, activation of adenylate cyclase enhances distension-induced ATP-release from the mouse bladder urothelium (3). In order to disclose the mechanism underlying the urothelium-derived modulation of β-AR- mediated relaxation of the human detrusor, we have examined whether adenylate cyclase or P2 purinoceptor is involved.

Study design, materials and methods

Human bladder tissue samples were obtained from non-cancer portions of the lateral bladder wall in 7 male patients (aged 67.9 ±3.7) undergoing radical cystectomy for bladder carcinoma. Exclusion criteria included previous pelvic radiotherapy, neoadjuvant chemotherapy or Bacillus Calmette-Guérin immunotherapy. Bladder strips (approximately 4mm x 7mm) were studied with or without urothelial layer. The muscle strips were pre-contracted with 0.1 μM endothelin-1 and relaxation was studied in response to the β-AR agonist, isoproterenol (10^{-10} M to 10^{-4}M). To exclude any α-AR-mediated processes, all experiments were carried out in the presence of the non-selective α-AR agonist, phentolamine (1 μM). Concentration–response curves for isoproterenol were obtained in the absence and presence of SQ 22536 (SQ; 100 μM), an adenylate cyclase inhibitor, or PPADS (30 μM), a non-selective P2 purinoceptor antagonist. At the end of each experiment 1 mM papaverine was used to determine maximum relaxation via cAMP increase as a reference. The t-test in a non-linear regression was used for comparison between strips with and without urothelial layer, and between the absence and presence of SQ or PPADS.

Results

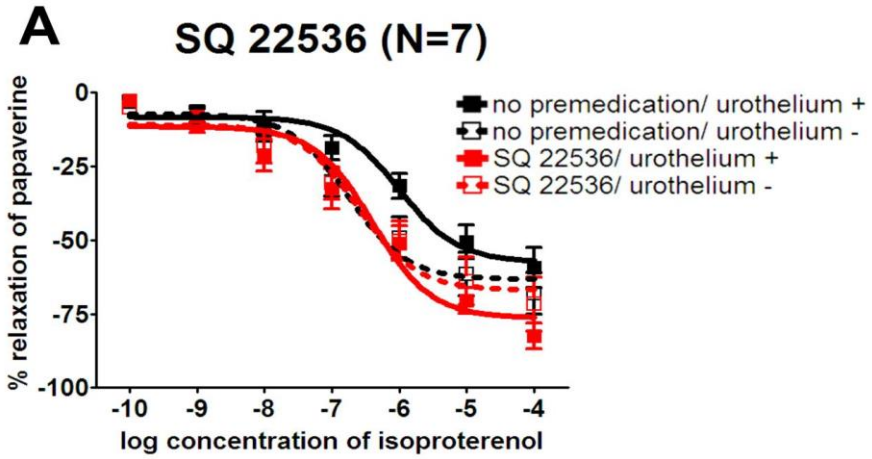
Isoproterenol relaxed human detrusor smooth muscle in a concentration dependent manner. isoproterenol had an effect of relaxing bladder strips at lower concentration in the strips without urothelial layer than in those with intact urothelial layer (pEC_{50} values: 6.75 vs 6.00, respectively; Figure 1A-B). The presence of 100 μM SQ 22536 significantly increased the maximum relaxation (Emax) in the mucosa-intact strips, whereas the presence of 100 μM SQ 22536 did not modified the concentration-response curve for isoproterenol in the strips without urothelial layer (Figure 1A). PPADS (30 μM) had a similar effect in the urothelium-intact strips (Figure 1B). The presence of 30 μM PPADS did not affect the isoproterenol-induced relaxation of the strips without urothelial layer (Figure 1A-B). In the presence of either of these drugs, there were no significant difference in pEC_{50} between the strips with and without urothelial layer (pEC_{50} values in the presence of SQ: 6.41 ±0.23 vs 6.40 ±0.17, pEC_{50} values in the presence of PPADS: 6.54 ±0.10 vs 6.60 ±0.18, respectively) (Figure 1A-B). PPADS did not modify the concentration-response curves for isoproterenol in the strips without urothelial layer (Figure 1A-B).

Interpretation of results

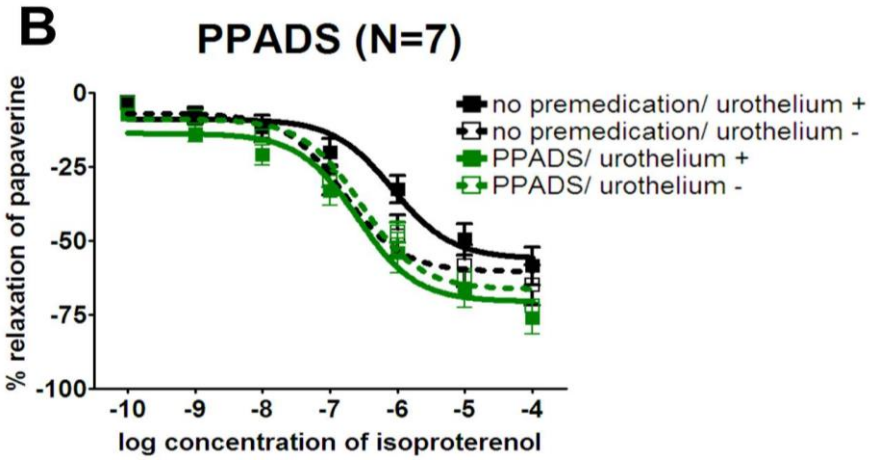
The result of the present study showed that the presence of intact urothelial layer reduces β-AR-mediated relaxation of human detrusor smooth muscle, which is in line with the findings previously reported, suggesting the involvement of a urothelium-derived inhibitory factor. The finding that SQ 22536 and PPADS eliminate the inhibitory action of the presence of intact urothelium indicates that the inhibitory action is mediated through activation of adenylate cyclase and P2 purinoceptors. As activation of adenylate cyclase in the urothelial cells enhances the distension-induced ATP-release from the urothelial cells themselves (3), it is conceivable that stimulation of β-ARs on the urothelial cells releases ATP via adenylate cyclase-dependent mechanism, which in turn inhibits detrusor relaxation. The negligible effects of SQ 22536 on relaxation in the denuded detrusor strips suggest that cAMP may not be directly involved in β-AR-mediated relaxation in human detrusor. Further studies are needed to clarify the relaxant inhibitor released from human urothelium and the mechanism of β-AR-mediated relaxation in human detrusor.

Concluding message

The present results indicate that the inhibitory effect of human urothelium on β-AR mediated relaxation involves with adenylate cyclase and P2 purinoceptors. These findings may give us a new insight into interaction between urothelium and detrusor in β-AR mediated relaxation.



	no premedication		*SQ 22536*	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
urothelium -	6.75±0.19	-63.3±3.6	6.41±0.23	-66.9±3.4
urothelium +	6.00±0.18*	-57.4±3.7	6.4±0.17	-76.3±3.9##



	no premedication		*PPADS*	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
urothelium -	6.81±0.19	-60.4±3.4	6.54±0.10	-66.4±1.9
urothelium +	6.07±0.19*	-56.0±3.7	6.60±0.18	-70.5±3.5##

Figure 1. Concentration-response curves of relaxation induced by isoproterenol in bladder strips with and with urothelial layer in the absence and presence of SQ22536, an adenylate cyclase inhibitor (A) and PPADS, a non-selective P2 purinoceptor antagonist (B).
*p<0.05: significant difference from the absence of urothelium
#p<0.05, ##p<0.01: significant difference from the no premedication

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Disclosures

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