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GUANYLATE CYCLASE AND P2X PURINOCEPTOR SIGNALLING PATHWAYS CONTRIBUTE TO UROTHELIUM-DERIVED INHIBITION OF B-ADRENOCEPTOR-MEDIATED RELAXATION IN HUMAN DETRUSOR SMOOTH MUSCLE

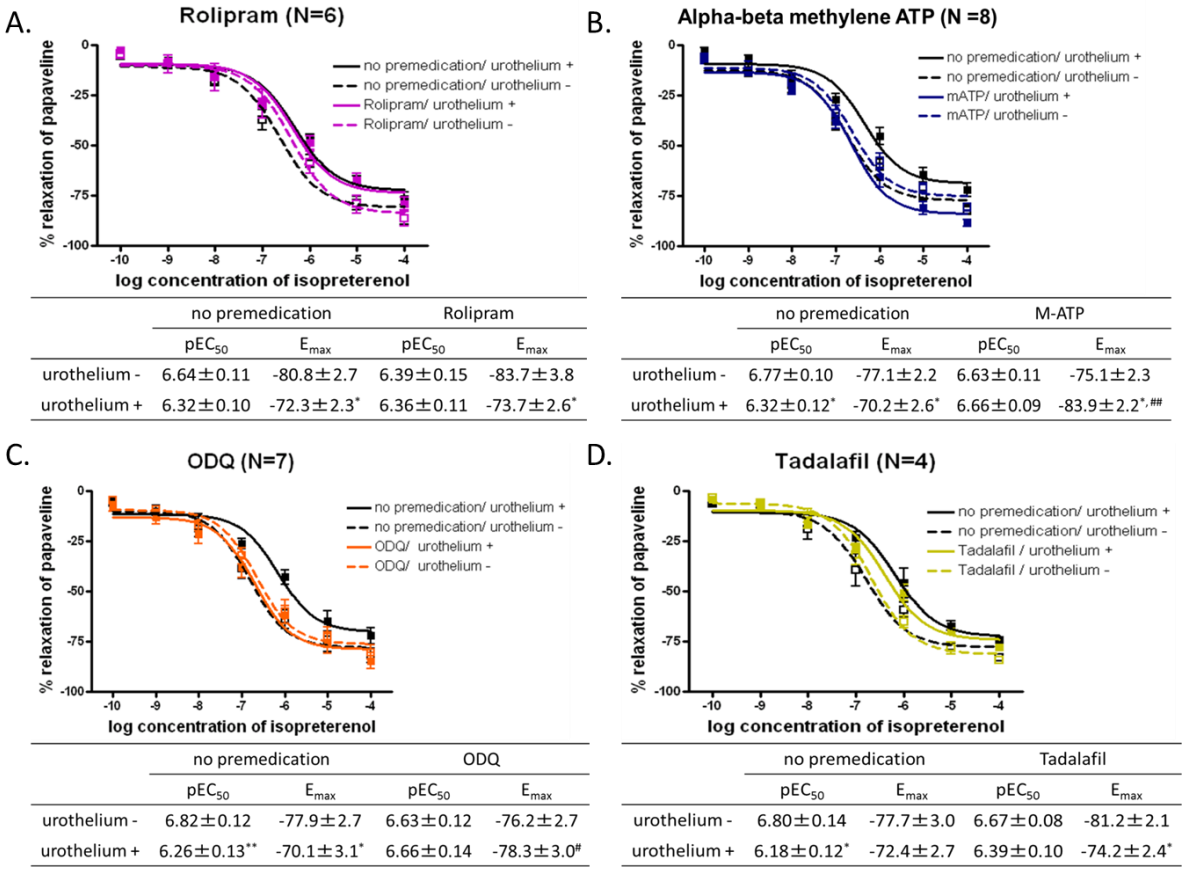
Hypothesis / aims of study
Althoughβ-adrenoceptors (β-ARs) is involved in the relaxation of detrusor smooth muscle, β-ARs have been also expressed in the urothelial cells, suburothelial interstitial cells, and nerve fibers. Previous studies demonstrated that activation of β-ARs in the urothelial cells releases nitric oxide (NO) (1), and also that urothelium inhibits β-AR-mediated relaxation in human detrusor smooth muscle via cAMP- and P2 purinoceptor-dependent mechanism (2). Moreover, another study demonstrated that activation of adenylate cyclase (AC) enhances distension-induced ATP-release from the mouse bladder urothelium (3). These previous findings suggest that nucleoside triphosphates such as ATP and GTP, and their second messengers (cAMP and cGMP) are closely related with the inhibitory action of the urothelium on β-AR-mediated relaxation of the detrusor. In this study, to disclose the mechanisms underlying the urothelium-derived inhibition of β-AR-mediated relaxation of the human detrusor, we evaluated effects of Pharmacological modulation of guanylate cyclase (GC), P2X purinoceptors and phosphodiesterase (PDE) signalling pathways on isoproterenol (ISO) induced relaxation of the human detrusor with and without urothelium.

Study design, materials and methods
Human bladder tissue samples were obtained from non-cancer portions of the lateral bladder wall in 19 male patients (aged 69.8 ± 1.6 years old) undergoing radical cystectomy for bladder carcinoma. Exclusion criteria included previous pelvic radiotherapy, neoadjuvant chemotherapy or Bacillus Calmette-Guérin immunotherapy. Bladder strips (approximately 4 mm x 7 mm) were studied with or without urothelium. To exclude any α-AR-mediated modulations, all experiments were carried out in the presence of a non-selective α-AR agonist, phentolamine (1 μM). The bladder strips were pre-contracted with 0.1 μM endothelin-1 and relaxant responses to ISO (10^{-10} to 10^{-4} M) were measured. At the end of each experiment, 1 mM papaverine was used to determine the maximum relaxation. Concentration–response curves for ISO were obtained in absence and presence of either one of the modulators of signalling pathways; rolipram (0.1 μM), a PDE4 inhibitor, α, β-methylene ATP (M-ATP: 10 μM x 5 times), a selective P2X purinoceptor agonist, ODQ (10 μM), a GC inhibitor, and Tadalafil (10 μM), a PDE5 inhibitor. The F-test in non-linear regression was used for comparison between strips with and without urothelium, and between the absence and presence of each premedication drug.

Results
ISO relaxed human detrusor smooth muscle in a concentration dependent manner, and this relaxant response to ISO in the urothelium-intact strips was weaker than that in the urothelium-denuded strips (Figure A-D). Compared with the absence of drugs, the presence of M-ATP or ODQ significantly increased the maximum relaxation (Emax) in the urothelium-intact strips, but not in the urothelium-denuded strips (Figure B, C). In contrast, the presence of rolipram or tadalafil did not affect Emax of ISO-mediated relaxation in the strips either with or without urothelium (Figure A, D). There were no significant differences in pEC_{50} among any group comparisons (Figure A-D).

Interpretation of results
The presence of urothelium reduced β-AR-mediated relaxation of human detrusor smooth muscle, suggesting the existence of urothelium-derived inhibitory actions on β-AR-mediated relaxation in the human detrusor smooth muscle. The results with M-ATP and ODQ suggest that activations of P2X purinoceptors and GC are involved in the urothelium-derived inhibitory actions. The contribution of P2X purinoceptors were in line with the previous study (2), but the present study is the first direct demonstration of the contribution of GC on β-AR-mediated relaxation in the human detrusor smooth muscle. In contrast, Neither rolipram or tadalafil affected the relaxant responses to ISO in the strips with or without urothelium, which suggests that neither PDE4 or PDE5 is likely to contribute to the urothelium-derived inhibitory actions on β-AR-mediated relaxation or to β-AR-mediated relaxation of the human detrusor smooth muscle itself.

Concluding message
The present results indicate that the inhibitory action of human urothelium on β-AR mediated relaxation involves GC and P2X purinoceptor signalling pathways. These findings may give us a new insight into interaction between urothelium and detrusor in β-AR mediated relaxation.



	no premedication		Rolipram	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
urothelium -	6.64±0.11	-80.8±2.7	6.39±0.15	-83.7±3.8
urothelium +	6.32±0.10	-72.3±2.3*	6.36±0.11	-73.7±2.6*

	no premedication		M-ATP	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
urothelium -	6.77±0.10	-77.1±2.2	6.63±0.11	-75.1±2.3
urothelium +	6.32±0.12*	-70.2±2.6*	6.66±0.09	-83.9±2.2$^{*,\#\#}$

	no premedication		ODQ	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
urothelium -	6.82±0.12	-77.9±2.7	6.63±0.12	-76.2±2.7
urothelium +	6.26±0.13**	-70.1±3.1*	6.66±0.14	-78.3±3.0$^{\#}$

	no premedication		Tadalafil	
	pEC_{50}	E_{max}	pEC_{50}	E_{max}
urothelium -	6.80±0.14	-77.7±3.0	6.67±0.08	-81.2±2.1
urothelium +	6.18±0.12*	-72.4±2.7	6.39±0.10	-74.2±2.4*

Figure. Concentration-response curves for isoproterenol in bladder strips with and without urothelial layer in absence and presence of rolipram (A), M-ATP (B), ODQ (C) and tadalafil (D).
$^{*}p<0.05$, $^{**}p<0.01$: significant differences from the urothelium-denuded strips
$^{\#}p<0.05$, $^{\#\#}p<0.01$: significant differences from the no premedication

References

1. Birder LA, Nealen ML, Kiss S, et al. Beta-adrenoceptor agonists stimulate endothelial nitric oxide synthase in rat urinary bladder urothelial cells. J Neurosci. 2002;22(18):8063-70.
2. Matsumoto-Miyai K, Yamada E, Yoshizumi M, et al. The regulation of distention-induced ATP release from urothelium by the adenylyl cyclase-cyclic AMP pathway. Biomed Res. 2012;33(3):153-7.
3. Osuka A, Shinbo H, Matsumoto R, et al. Expression and functional role of beta-adrenoceptors in the human urinary bladder urothelium. Naunyn Schmiedebergs Arch Pharmacol. 2008;377(4-6):473-81.

Disclosures

Funding: None **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Ethics Committee, The University of Tokyo Graduate School of Medicine **Helsinki:** Yes **Informed Consent:** Yes