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ROLE OF UROTHELIUM ON (3-ADRENOCEPTOR MEDIATED RELAXATION IN HUMAN DETRUSOR MUSCLE

Hypothesis / aims of study

Urothelium of the urinary bladder not only serves as a barrier protecting the underlying smooth muscle against irritating urine constituents, but also provides significant inhibitory effects on detrusor contractions in response to various stimulations.[1] Catecholamine-mediated relaxation of human detrusor is exclusively mediated via β_3-adrenoceptors (β_3-AR).[2,3] Recently we have shown that human detrusor relaxes in response to β_3-adrenoceptor (β_3-AR) stimulation with (-)-norepinephrine and that the presence of urothelium significantly reduces the agonist's potency without directly interfering with β_3-AR function. In order to investigate the mechanisms underlying the urothelium-induced modulation of β_3-AR-mediated relaxation, we have examined whether muscarinic receptors or prostaglandines are involved.

Study design, materials and methods

Human detrusor tissue samples were obtained from patients undergoing radical cystectomy for the treatment of muscle invasive bladder cancer. Detrusor strips from 12 male and 10 female patients (mean age 69 years) were studied with and without an intact mucosa layer. Muscle strips were pre-contracted with either 1 µM carbachol (CCh) or 40 mM KCl and relaxation was studied in response to the β-AR agonists (-)-norepinephrine and (-)-isoproterenol. To exclude any α-AR mediated processes all experiments were carried out in the presence of the non-selective α-AR antagonist's phentolamine (3 µM) and prazosin (1 µM). The unselective cyclooxygenase (COX) inhibitor diclofenac was used to block prostaglandine synthesis, muscarinic receptors were blocked with atropine, and involvement of β_3-AR was verified with the selective β_3-AR antagonist L748,337. At the end of each experiment 10 µM forskolin was used to determine maximum relaxation via cAMP release.

Results

(-)-Norepinephrine and (-)-isoproterenol relaxed human detrusor muscle in a concentration dependent manner. (-)-Norepinephrine was more potent and effective in relaxing both CCh- or KCl-precontracted detrusor strips in the absence of urothelium than in its presence. In contrast, (-)-isoproterenol was only marginally more effective without any difference in potency (Table 1).

Table 1: β-AR induced human detrusor relaxation in the presence and absence of urothelium

	Drug	Uro-thelium	Precontraction CCh (1 µM)			Precontraction KCl (40 mM)		
			n	$logEC_{50}$ [M]	E_{max} [%]	n	$logEC_{50}$ [M]	E_{max} [%]
NE	control	+	15/9	-5.5 ± 0.2	58 ± 3	6/6	-6.2 ± 0.1	59 ± 6
		-	15/11	-6.3 ± 0.0**	67 ± 2**	6/6	-6.5 ± 0.1**	86 ± 3**
	+1 µM Atropine	+				6/6	-6.1 ± 0.1	65 ± 4
		-				6/6	-6.6 ± 0.1**	86 ± 2***
	+100 µM Diclofenac	+	8/4	-6.2 ± 0.2	71 ± 4			
		-	8/4	-6.6 ± 0.0*	77 ± 6**			
Iso		+	7/7	-6.6 ± 0.1	50 ± 6	7/7	-6.7 ± 0.2	50 ± 6
		-	9/9	-6.6 ± 0.1^{ns}	63 ± 2^{ns}	11/11	-6.7 ± 0.1^{ns}	63 ± 5^{ns}

NE – (-)-norepinephrine; *Iso* – (-)-isoproterenol

* $p < 0.05$; ** $p < 0.01$; ** $p < 0.01$; *** $p < 0.001$; ^{ns} – not significant

The presence of 1 µM atropine in KCl-precontracted detrusor muscle did not influence the (-)-norepinephrine-induced relaxation in denuded or intact detrusor muscle. However, 100 µM diclofenac shifted the concentration-effect curve for (-)-norepinephrine to lower concentrations and increased maximum relaxation (Table 1). Those effects were independent of the presence of urothelium. Increasing concentrations of the selective β_3-AR antagonist L748,337 (0.01 – 1 µM) shifted the concentration-effect curve for (-)-norepinephrine to higher concentrations without affecting maximum relaxation. Calculation of apparent affinity (pK_B[M]) for L748,337 using Schild plot analysis (slope=1) yielded 7.66 ± 0.10 for urothelium-denuded and 7.75 ± 0.19 for detrusor strips with an intact urothelium.

Interpretation of results

Catecholamine-induced relaxation of human detrusor is exclusively mediated through β_3-AR. The presence of an intact urothelium reduces potency and efficacy of (-)-norepinephrine indicating involvement of urothelium-mediated processes not only during detrusor contraction but also during relaxation. Lack of the influence of atropine on the relaxation response to (-)-norepinephrine in KCl-precontracted preparations with and without urothelium indicates that acetylcholine release is not activated. On the other hand, the shift by COX-inhibitors, suggests that prostaglandines are involved in the β-AR-mediated relaxation process. However, the urothelium-induced modulation seem not to include a prostaglandine-sensitive mechanism, because effects of diclofenac were independent of the presence of urothelium. Apparent affinity of the selective β_3-AR antagonist

L748,337 is unchanged. The surmountable antagonism and similar pK_B-values of L748,337 in denuded and intact detrusor strips indicate that relaxation is mediated by the β_3-AR subtype under both conditions.

Concluding message

In human detrusor, β_3-ARs play the key role during catecholamine-mediated relaxation. The presence of urothelium changes potency and maximum relaxation of (-)-norepinephrine but does not significantly influence potency of (-)-isoproterenol. Our results suggest that during catecholamine-induced relaxation the urothelium releases a contraction-enhancing factor. The experiments with diclofenac and atropine indicate that neither prostaglandines nor muscarinic receptor stimulation contribute to this putative enhancing factor. Nevertheless prostaglandines affect the β_3-AR mediated relaxation which could be explained by chronic inflammation in the cancerous urinary bladder.

References

1. Br J Pharmacol (2000) 129(3): 416-419
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Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**