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A MOLECULAR BIOLOGICAL AND FUNCTIONAL ANALYSIS OF B-ADRENOCEPTORS IN HUMAN DETRUSOR SMOOTH MUSCLE AND UROTHELIUM

Hypothesis / aims of study

Recent studies suggest that urothelial cells express various receptors, and release neurotransmitter to stimulate suburothelial afferent nerves or *via* myofibroblasts[1]. Although β-adrenoceptors (ARs) are abundant in detrusor smooth muscle and play important roles in bladder relaxation during urinary storage, there is little evidence about molecular biological and functional analysis of β-ARs in human urothelium. Thus, this study was conducted to examine whether mRNAs of β-ARs were expressed in human urothelium as well as detrusor smooth muscle, and whether stimulation of urothelial β-ARs modulated relaxant effects on human detrusor strips. Furthermore, we also investigated whether nitric oxide released from urothelium, which was evoked by β-AR agonists, modulated relaxant effects on human detrusor strips.

Study design, materials and methods

The human bladder specimens were obtained from patients undergoing total cystectomy. After connecting tissues had been removed, the specimens were cut into a pair of detrusor muscle strips with or without an intact urothelium (approximately 15×5×5mm) for functional studies. Total RNA was extracted from detrusor smooth muscle and urothelium, and expression of β_1-, β_2- and β_3-AR mRNA in detrusor muscle and urothelium was determined by reverse transcription polymerase chain reaction (RT-PCR). Sequences of sense and anti-sense oligonucleotides for β_1-, β_2- and β_3-AR were used according to the previous report[2]. Detrusor strips were suspended in 10mL organ baths containing pre-gassed Krebs solution. A resting tension of the detrusor strip was 1.0g and isometric tension was recorded. After phentolamine (1μM) was applied, concentration-response curves to isoproterenol were constructed. In some detrusor strips with an intact urothelium, concentration-response curves were performed in the presence of L-NAME (100μM). Forskolin (10μM) was added to obtain maximal relaxation of the detrusor muscle. The maximal relaxant responses and pEC_{50} values were expressed as a mean ± SEM, and Student's *t*-test was used for statistical analysis.

Results

The expression of β-adrenoceptor mRNA in human detrusor smooth muscle and urothelium was examined using RT-PCR (n=5). PCR products for β1-, β2- and β3-adrenoceptor were detected in all the preparations of the human detrusor smooth muscle and urothelium, and the expected size of PCR products were 265, 329 and 314 bp, respectively. PCR product for β-actin as an internal standard was also detected in all preparations and its expected size was 353 bp, furthermore, PCR products without prior RT of the RNA did not reveal any positive bands. The specificity of the primers and the authenticity of the corresponding PCR products were verified by digestion of PCR products with specific restriction enzymes. β1-adrenoceptor PCR products were digested with Rsa I (generated fragments of 67 and 198 bp); β2-adrenoceptor with Rsa I (93 and 236 bp); and β3-adrenoceptor with Apa I (123 and 191 bp).

Isoproterenol produced a concentration-dependent relaxation of the urothelium-denuded detrusor strips with a pEC_{50} of 6.31 ± 0.23 and a maximum relaxation of 84.2 ± 3.4 % (n=7). In the urothelium intact detrusor strips, Isoproterenol also produced a concentration-dependent relaxation with a pEC_{50} of 5.58 ± 0.30 and a maximum relaxation of 80.4 ± 3.2 % (n=9). The relaxant response to isoproterenol of the urothelium intact detrusor strips was partially lower than that of the urothelium-denuded detrusor strips ($p < 0.05$). However, L-NAME did not have any significant effects on relaxant responses of detrusor strips with an intact urothelium compared to the control (n=5).

Interpretation of results

These results provided the first evidence for the existence of the β-AR subtypes mRNA in human urothelium as well as detrusor smooth muscle. In addition, urothelial β-ARs may take a part in the inhibitory effect on detrusor relaxation evoked by β-AR agonists. However, nitric oxide does not influence on this inhibitory effect.

Concluding message

This study suggested that β-AR agonists might stimulate the release of an unidentified inhibitory factor from human urothelial β-ARs to suppress detrusor relaxation. However, the inhibitory factor was distinct from nitric oxide. Further work may be necessary to confirm the function of urothelial β-ARs in humans.

References

1 Urology (2004) 64; 7-11.
2 J Clin Invest (1993) 91; 344-9.

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