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EXPRESSION AND DISTRIBUTION OF ALPHA1-ADENOCEPTOR SUBTYPES IN HUMAN UROTHELIUM, SUBUROTHELIUM AND DETRUSOR MUSCLE OF THE URINARY BLADDER.

Hypothesis / aims of study

Urothelial cells express adrenergic and muscarinic receptors. It is common knowledge that these cells release neurotransmitters such as acetylcholine, nitric oxide, adenosine triphosphate (ATP) to stimulate suburothelial afferent nerves or *via* myofibroblasts. Recent experimental findings show that urothelial **α**$_1$-adrenoceptor (AR) modulates the bladder function. Clinically, **α**$_1$- AR antagonist have been found to relieve the irritative bladder symptom in men with BPH or women. However, this mechanism has been still under investigation. In humans, there is little direct evidence whether **α**$_1$-AR subtypes exists in the human urinary bladder urothelium and suburohtelium. Therefore, we investigated the expression of **α**$_{1A}$ and **α**$_{1D}$ - AR subtypes in human urinary bladder urothelium and detrusor smooth muscle by reverse transcription-polymerase chain reaction (RT-PCR), in addition, we examined the distribution of **α**$_{1A}$ and **α**$_{1D}$ - AR subtypes in human urinary bladder urothelium, suburothelium and detrusor smooth muscle by immunohistochemistry.

Study design, materials and methods

The human bladder specimens were obtained from patients undergoing total cystectomy.

The tissues were composed of full-thickness sections of the human urinary bladder taken from macroscopically and histologically normal areas of specimens removed for bladder carcinoma. Total RNA was extracted from detrusor smooth muscle and urothelium, and expression of **α**$_{1a}$ and **α**$_{1d}$ - 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 **α**$_{1a}$ and **α**$_{1d}$ - AR were used according to the previous report. PCR products (8 μl) were visualized by electrophoresis on 3.0% agarose gels with ethidium bromide. To rule out the possibility of amplifying genomic DNA, PCR was performed with no prior RT of the RNA in all experiments.

For immunohistochemistry, paraffin sections (4 μm) of archival human surgical resection specimens of the urinary bladder were immunostained using the HistoFine SimpleStain MAX-PO KIT (Nichirei Bioscience, Tokyo, Japan), and incubated with primary antibodies for **α**$_{1A}$ and **α**$_{1D}$ -adrenoceptor (polyclonal rabbit antibody, Santa Cruz Biotechnology, California, USA). All sections were counterstained with Mayer's haematoxylin, and mounted under coverslips. For each section, a negative control consists of no primary incubation and a positive control was performed with human surgical resection specimens of the prostate. Immunohistochemical staining of **α**$_{1A}$ and **α**$_{1D}$ - AR was recorded as positive or negative. A sample was considered negative when immunostaining of the cells were the same as the negative control.

Results

The expression of **α**$_1$-AR mRNA in human detrusor smooth muscle and urothelium was examined using RT-PCR. PCR products for **α**$_{1a}$ and **α**$_{1d}$ - AR were detected in all the preparations of the human detrusor smooth muscle and urothelium, and the expected size of PCR products were 343 and 150 bp, respectively. The distribution of **α**$_{1A}$ and **α**$_{1D}$ -AR, as defined by positive staining, was observed in the human urinary bladder urothelial cells and suburothelial cells of all the sections by immunohistochemistry. Positive staining for **α**$_{1A}$ and **α**$_{1D}$ -adrenoceptor was also identified in the detrusor smooth muscle cells. The negative control gave no staining of urothelial cells, suburothelial cells and detrusor smooth muscle cells in any of the sections.

Interpretation of results

These results provided the first evidence for distribution of **α**$_{1A}$ and **α**$_{1D}$ -AR in human bladder urothelium and suburothelium by immunohistochemistry. According to these results of RT-PCR, the existence of **α**$_{1A}$ and **α**$_{1D}$ -AR in human bladder urothelium was detected as well as detrusor muscle.

Concluding message

Our results show the existence of **α**$_{1A}$ and **α**$_{1D}$ -AR in human bladder urothelium and suburothelium. In rats, endogenous catecholamines act on **α**$_{1D}$ – AR in the urothelium to facilitate mechanosensitive bladder afferent nerve activity and reflex voiding.[1] The other finding shows that norepinephrine releases nitric oxide from the urinary bladder epithelium.[2] Further, **α**$_{1D}$ - AR antagonist (naftopidil) inhibit the rat bladder activity due to blocking ATP release from the bladder urothelium *in vitro*.[3] Our results may give rise to the consumption that urothelial and suburothelial **α**$_1$-ARs modulate the micturition reflex in humans; however, we should perform additional functional *in vitro* study using human specimens.

References

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