

THE DIFFERENTIAL EXPRESSION OF P1 RECEPTORS IN THE HUMAN BLADDER.

Hypothesis / aims of study

In stable human detrusor smooth muscle, contractile activation is mediated solely by acetylcholine, as the contractions are completely abolished by atropine. However in the overactive bladder, atropine resistant contractions can be evoked. Pre-treatment of these samples with α,β -methylene-ATP, a non-hydrolyzable analogue of ATP, removes this atropine resistant component, implying that ATP is also acting as a neurotransmitter in the bladder (1). ATP acts through P2 receptors of the purinergic (P) receptor pathway. Adenosine, a breakdown product of ATP, acts through P1 receptors (2). It is known that human detrusor smooth muscle expresses P1 receptors, but the role of adenosine in detrusor contraction is poorly understood. Furthermore, characterization of the relative proportions of the four P1 receptor subtypes (A_1 , A_{2A} , A_{2B} , A_3) has not been determined. Our aim was to examine subtype distribution in the human bladder through mRNA expression.

Study design, materials and methods

Detrusor specimens stripped of mucosa and serosa, were obtained from 20 patients undergoing cystectomy or augmentation ileocystoplasty operations with local ethical committee approval and informed patient consent.

Total RNA was extracted from these specimens using a phenol chloroform procedure. This RNA was reverse transcribed (RT) using oligonucleotides and the resulting mRNA transcripts were amplified by the Polymerase chain reaction (PCR), using gene specific primers. The PCR protocol was: Denaturation at 94°C for 1 minute, Annealing at 56°C for 45 seconds and Elongation at 72°C for 45 seconds. This was repeated for 35 cycles, followed by extension for 10 minutes. Total RNA was also subjected to RT-PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH-3) which was used as the housekeeping gene. The positive control was RNA extracted from human ileum, which we have shown previously to express P1 receptors. The PCR products were analysed electrophoretically on a 1% agarose gel, followed by scanning densitometry (measuring optical density, OD), using Labworks Image Acquisition Analysis software. The ratio of P1 receptor OD: GAPDH-3 OD was used to determine the level of P1 receptor mRNA transcription.

Results

In comparison with GAP expression in the same tissues, it seems that A_{2B} and A_3 receptor mRNA is strongly expressed in **all** bladder specimens. A_{2A} , and to a greater extent, A_1 receptor expression was variable; with A_1 expression being significantly down regulated as compared to the expression of the A_{2B} and A_3 receptors.

Interpretation of results

The four adenosine receptor subtypes in the human bladder are not expressed equally.

Concluding message

The question arises whether P1 receptor subtype expression is homogenous in bladders of differing pathologies. It would be interesting to correlate P1 receptor subtype expression with detrusor overactivity and neurogenic bladder dysfunction, and compare it with receptor expression in stable bladders. At present, we are in the process of investigating these potential differences further.

References

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2. Differential cotransmission in sympathetic nerves: role of frequency of stimulation and prejunctional autoreceptors. (1999). *J.Pharmacol Exp Ther* **290**:241-246.

