

SLOWING DOWN ADENOSINE FORMATION FROM RELEASED ATP CONTRIBUTES TO CHOLINERGIC NERVE HYPERACTIVITY IN THE OBSTRUCTED HUMAN BLADDER

Hypothesis / aims of study

Overactivity of the human detrusor due to benign prostatic hyperplasia (BPH) causes significant lower urinary tract symptoms. Contractions of normal human bladder are almost exclusively mediated by acetylcholine (ACh), via muscarinic M₃ receptors. However, ATP acting via P₂ purinoceptors located on sensory nerve afferents and detrusor smooth muscle gains a significant relevance in obstructed patients. Recently, we provided evidence demonstrating that urinary ATP from patients with detrusor overactivity is statistically higher than age-matched controls. Determination of a high AUC (83,2%, CI95%: 69,7-96,7) is consistent with urinary ATP being a highly sensitive biomarker of detrusor overactivity, showing no correlation with voided urine volume, urinary creatinine and urinary lactate dehydrogenase (LDH) activity. Our group also showed that bladder strips from BPH patients exhibit a 3-fold increase in nerve-evoked ATP release as compared to controls. The role of ATP may, however, be complicated by compartmentalization of purinoceptors together with ecto-NTPDases at cell surface microdomains. Previous findings demonstrated that the detrusor of BPH patients exhibit a slow inactivation kinetic of ATP into biologically-active derivatives, namely adenosine, as compared to cadaveric controls, which might contribute to increase the ATP-mediated tonus in the bladder. This, prompted us to investigate whether slowing down adenosine formation from the catabolism of released ATP could also contribute to up-regulate the release of the principal neurotransmitter, ACh, in obstructed human bladder samples.

Study design, materials and methods

Human bladder samples were collected from patients with outflow obstruction submitted to transvesical prostatectomy and from cadaveric organ donors (control subjects). Detrusor strips were mounted in organ baths and were superfused with oxygenated Tyrode's solution at 37°C. The release of [³H]ACh from detrusor strips loaded with [³H]choline was induced by electrical field stimulation (10 Hz, 200 pulses). The kinetics of extracellular ATP (30 μM) hydrolysis and adenosine formation was evaluated by HPLC. Immunolocalization studies of P₂ receptor and ecto-NTPDase subtypes were performed by laser scanning confocal microscopy (Olympus, FV1000).

Results

The extracellular hydrolysis of ATP (30 μM) and subsequent adenosine formation was significantly ($P < 0.05$) slower ($t_{1/2} = 41 \pm 7$ min, $n = 4$) in obstructed human bladders than in controls ($t_{1/2} = 30 \pm 6$ min, $n = 3$). Impairment of the nucleotidase activity in overactive bladder samples was positively correlated with a significant loss of immunoreactivity against NTPDases 1, 2 and 3 in the detrusor from BPH patients shown by confocal microscopy. Blockade of P_{2X3} receptors located on VAcHT-positive nerve fibres with TNP-ATP (10 nM) reduced [³H]ACh release by 27±9% ($n = 4$) in controls and by 43±6% ($n = 4$) in obstructed detrusor samples. A similar pattern was observed using apyrase (2U/ml), the enzyme that converts ATP directly into AMP, which may be subsequently hydrolysed into adenosine by ecto-5'-nucleotidase. Inactivation of endogenous adenosine with adenosine deaminase (0.5 U/ml) failed to modify the release of [³H]ACh from stimulated bladder samples from BPH patients and cadaveric controls. Inhibition of evoked [³H]ACh release by endogenous adenosine was only apparent upon blocking the uptake and extracellular deamination of the nucleoside with dipyridamole (0.5 μM) and EHNA (50 μM), respectively. Interestingly, activation of adenosine A₁ receptors with R-PIA (300 nM), but not of A_{2A} receptors with CGS21680C (3 nM), decreased [³H]ACh release by 30±8% ($n = 6$) and by 45±1% ($n = 4$) in control and BPH patients, respectively. These results suggest that A₁ receptors negatively modulate cholinergic nerve activity and that these receptors may be up-regulated in obstructed bladders.

Interpretation of results

Data suggest that increases in tissue bioavailability of ATP in patients with BPH, due to ATP release from the bladder urothelium and/or nerve terminals together with reduced extracellular inactivation of the nucleotide, might contribute to cholinergic nerve hyperactivity yielding to the detrusor overactivity. In addition, it seems like adenosine may exert a tonic inhibitory effect regulating ACh release from cholinergic nerve terminals, which might be unbalanced in BPH patients as compared with cadaveric controls. In view of the reduced levels of endogenous adenosine originated from released ATP in BPH patients, inhibitory adenosine A₁ receptors may be highly sensitive (up-regulated) to R-PIA in BPH patients.

Concluding message

In conclusion, the global impairment of ecto-NTPDase activity in overactive bladder samples leading to enhanced bioavailability of ATP and decreased formation of inhibitory adenosine may contribute to detrusor overactivity in BPH patients. This may be partially overcome upon increasing endogenous adenosine accumulation in the detrusor by blocking the nucleoside inactivation pathways, both uptake and extracellular deamination, which may also benefit from up-regulation of adenosine A1 receptors.

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<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	HUMAN
<i>Was this study approved by an ethics committee?</i>	Yes
<i>Specify Name of Ethics Committee</i>	The procedures were all approved by the Ethics Committees of Centro Hospitalar do Porto (University Hospital) and of Instituto de Ciências Biomédicas de Abel Salazar (Medical School) of the University of Porto. The investigation conforms to the principles outlined in the Declaration of Helsinki.
<i>Was the Declaration of Helsinki followed?</i>	Yes
<i>Was informed consent obtained from the patients?</i>	Yes