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ATP RELEASE FROM THE UROTHELIUM MEDIATES BLADDER EXCITATION TRIGGERED BY UDP-SENSITIVE P2Y6 RECEPTORS

Hypothesis / aims of study

Bladder distension, inflammation and chemical irritation stimulate the release of adenine and uracil nucleotides (*e.g.* ATP and UTP) from the bladder epithelium, which are thought to play important roles in bladder physiological and pathological conditions acting on ionotropic P2X and metabotropic P2Y receptors. $P2X_1$ receptors are found on the detrusor smooth muscle, whereas receptors containing the $P2X_3$ subunit are found on pelvic nerve afferent nerves implicated in the micturition reflex triggered by bladder filling. Thus, when administered intravesically, ATP increases bladder activity in the rat. ATP stimulation of micturition may be partially counteracted by its catabolism into ADP by ecto-NTPDases at the urinary bladder; ADP acts through inhibitory $P2Y_1$ receptors. In contrast to the compelling evidence for the extracellular signalling role of ATP, the hypothesis that uridine nucleotides may also fulfill an autocrine/paracrine role has only recently gained experimental support. Metabotropic $P2Y_2$ recognizes both ATP and UTP as the most potent agonists, while $P2Y_4$ and $P2Y_6$ receptors were recently identified and characterized as UTP- and UDP-selective receptors in humans, respectively.

Study design, materials and methods

In this study, we investigated the role of UDP on the micturition reflex triggered by bladder distension (intravesical saline infusion 2.4 ml/h) in urethane-anaesthetized male Wistar rats (300−450 g). We compared *in vivo* cystometric assays with *in vitro* myographic recordings; UDP (100 µM) was applied either through a cannula inserted in the bladder dome or directly to the bathing solution outside the bladder. We also evaluated the effect of UDP on $[^3H]$-ACh release evoked by electrical field stimulation (10 Hz, 200 pulses, 0.2 ms, 50 V) on bladder strips with and without the urothelium.

Results

Intravesical UDP (100 µM) increased the voiding frequency (VF) by 45±8% (*n*=12), without affecting the amplitude (A) and the duration (Δt) of bladder contractions. This effect was significantly ($P<0.05$) attenuated by intravesical MRS2578 (100 nM, a $P2Y_6$ selective antagonist). UDP was unable to cause bladder contractions *in vitro*. Intravenous perfusion with A317491 (100 nM, a selective $P2X_3$ antagonist) prevented UDP-induced increases in the VF. The ecto-ATPase inhibitor, ARL67156 (200 µM), enhanced the VF in the rat *in vivo* and prevented UDP from increasing bladder activity when it was applied inside the bladder. Intravenous MRS2179 (300 nM, a $P2Y_1$ selective antagonist) decreased the VF caused by UPD; this effect was mostly due to a significant ($P<0.05$) increase in the duration (Δt) of bladder contractions. UDP (100 µM) significantly decreased evoked $[^3H]$-ACh release in the presence of urothelium, but it was devoid of effect in its absence. MRS2578 (100 nM) prevented transmitter release-inhibition caused by UDP. UDP inhibition was transformed into a significant facilitatory effect by MRS2179 (300 nM).

Interpretation of results

Data indicate that UDP-mediated increases in the voiding frequency are mediated through urothelium and requires intact nervous circuitry. Expression of $P2Y_6$ receptors in the urothelium and $P2X_3$ receptors in suburothelial nerve fibres were confirmed by immunocytochemistry. We, therefore, hypothesized that UDP excitation results from stimulation of urothelial $P2Y_6$ receptors, which indirectly trigger ATP release and the subsequent activation of $P2X_3$ receptors on suburothelial sensory nerve fibres. UDP-induced bladder excitability may be partially counteracted through ADP formation from released ATP via ecto-ATPase (or NTPDase 2), which was detected together with NTPDase 1 and 3 on urothelial and suburothelial layers by confocal microscopy. Endogenous ADP acting through $P2Y_1$ receptors restrains ACh release from stimulated cholinergic motoneurons, indirectly reducing smooth muscle activity.

Concluding message

In conclusion, this study emphasizes the complexity of purinergic signalling in the bladder. Membrane compartmentalization of ecto-enzymes and other components of purinergic signaling cascade have been demonstrated. Thus, nucleotide-inactivating ecto-enzymes are located in close proximity with each other and next to nucleotides permeation sites, releasable stores and purinoceptors. It is, therefore, reasonable to accept that released extracellular nucleotides (e.g. ATP and UTP) and their derivatives are mainly concentrated on specific microenvironments where divergent cellular functions ($P2X_3$-mediated excitation and $P2Y_1$-inhibition) may take place. These multiple mechanisms for extracellular accumulation of particular P2 nucleotide

agonists underscore why the characterization of potential nucleotide release mechanisms in a particular tissue or cell model usually requires a corresponding analysis of extracellular nucleotide metabolism in that cell system.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Animal handling and experiments followed the guidelines defined by the European Communities Council Directive (86/609/EEC).**