231

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EFFECT OF ESCHERICHIA COLI LIPOPOLYSACCHARIDE ON STRETCH-INDUCED ATP RELEASE FROM CULTURED UROTHELIAL CELLS.

Hypothesis / aims of study

Urinary tract infection (UTI), commonly caused by *Escherichia coli*, is experienced by 50% of all women at least once during their lifetime. The symptoms of UTI include increased voiding frequency and urgency, usually accompanied by a sensation of burning pain and foul smelling urine. ATP released from the bladder urothelium is an important molecule for signaling urinary urgency. Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria and triggers the induction of host immune responses. LPS is made up of a highly conserved core and lipid A together with a surface-exposed O antigen. There are over 180 different O antigen types produced by different *E. coli* strains. The aim of the current study was to examine the effect of LPS from *E. coli* on stretch-induced ATP release from human urothelial cells in culture.

Study design, materials and methods

Human urothelial RT4 cells were cultured at 37^{o}C with 5%CO_2 in 24-well plates in McCoys 5A culture media (supplemented with foetal bovine serum, glutamate and antibiotics). RT4 urothelial cells are a cell line from a benign urothelial papilloma. At confluence cells were treated with (10µg/mL) *E. coli* LPS. After 24h cells were incubated for 10min with Krebs as control or hypotonic Krebs solution (50%) to mimic stretch. Supernatant was collected and the ATP concentration measured using a bioluminescence assay. Results are presented as nM ATP released [median (IQR)]. Two different LPS types were used and cells pre-treated with the different LPS types were compared using a non-parametric Kruskal-Wallis test.

Results

Results of experiments examining the effect of LPS pre-treatment on control and stretch-induced ATP release are shown in Table 1. Control ATP release was unaffected by LPS pre-treatment. Hypotonic media (stretch) induced an increase in ATP release [n=16, P<0.0001]. This was not affected by pre-treatment with LPS from *E. coli* O111:B4 [n=8]. In contrast, pre-treatment with LPS from *E. coli* O55:B5 reduced stretch-induced ATP release such that it was not significantly greater than release in the control cells [n=8, P<0.001].

Table 1: Effect of LPS pre-treatment on ATP release from urothelial cells

E. coli LPS type	Control ATP release	Stretch induced ATP release
none	14.2nM (7.2-20.7) n=16	50.8nM (22.4-64.9) n=16
O55: B5	12.9 (7.9-17.6) n=8	20.4nM (18.4-29.8) n=8
O111: B4	16.7nM (8.2-27.9) n=8	52.9nM (28.5-83.4) n=8

Interpretation of results

Stretch-induced ATP release was significantly reduced following exposure of urothelial RT4 cells to LPS of O55:B5 type but not O111:B4 type. These findings demonstrate that the effect of LPS on stretch-induced ATP release is differs according to LPS type.

Concluding message

The decrease in stretch-induced ATP release by O55:B5 but not by O111:B4 *E. coli* LPS suggests that infection by uropathogens with different LPS serotypes may result in different urothelial purinergic signaling in response to filling. This could have important implications to our understanding of the etiology of urgency associated with UTI. In light of these results, the effect of LPS on stretch-induced ATP release from serotypes common to uropathogenic *E. coli* strains should be examined.

Disclosures

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