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BIG POTASSIUM CHANNEL (BK) ACTIVITY IN FEMALE MOUSE BLADDER UMBRELLA CELLS IS ENHANCED BY BACTERIAL LIPOPOLYSACCHARIDE: AN ACUTE HOST RESPONSE IN UTI PATHOGENESIS

Hypothesis / Aims of study

The bladder urothelium is a multi-layered epithelium composed of umbrella, intermediate and basal cell layers, with the umbrella cells providing barrier and protective function against uropathogens. Current studies of urothelial cell function use enzymes to dissociate cells or utilize cultured urothelial cells grown as a monolayer which cannot recapitulate the differential function of urothelial cells within a stratified epithelium. Lipopolysaccharide (LPS) is a component of the outer membrane of gram-negative uropathogenic *E. coli*, which is the main cause of female urinary tract infection (UTI). LPS has been used as a surrogate for *E. coli* in study of host response to infections. Our aims of this study are to: (1) introduce a non-enzymatic method to dissect pure urothelium without lamina propria attached, (2) apply molecular biology and patch clamp techniques to study mouse umbrella cell's potassium (K) currents in response to LPS.

Study design, methods and Materials

Female C57BL/6 mice 8-12 weeks of age were used. The mouse bladder was opened and cut into strips. First, mucosa (urothelium + lamina propria) was separated from detrusor from each strip. Second, the mucosa was further peeled into a pure urothelial sheet and lamina propria by forceps under microscope. A small urothelial sheet was placed luminal-side down and umbrella cells were exposed after removing basal and intermediate cells with a glass micropipette (Fig. 1). The dissected urothelial sheets, lamina propria and detrusor were used in RT-PCR mRNA expressions for BK and LPS receptors (TLR4, CD4, MD-2). Immunofluorescence with anti-BKα subunit antibody was performed in full thickness bladder sections. Potassium currents in the basal membrane of the umbrella cells were measured by cell-attached and inside-out configurations. 2-3 patches were performed from each bladder and each experiment included 6-10 patches. Electrode pipette solution contained: 140mM KCl and 1.8mM $MgCl_2$ and 10mM Hepes. Bath solution #1 contained: 140mM NaCl, 5mM KCl, 1mM $CaCl_2$, 1mM $MgCl_2$, 5mM glucose and 10mM Hepes. Bath solution #2 contained: 140mM KCl, 10mM NaCl, 1mM $CaCl_2$, 0.8mM $MgCl_2$, 5mM glucose and 10mM Hepes. For calcium dose-response experiments, 1mM EGTA was added to bath solution #2 with Ca concentrations were calculated by CaEGTA calculator. Plymyxin B and H89 were used to treat urothelial sheets prior to LPS exposure. Clampfit 10.2 software was used to analyse patch clamp data. Channel activity defined as NPo (N = numbers of open channels; Po = single channel open probability). Differences between groups (mean ± SE) were tested by one-way ANOVA and $p < 0.05$ was considered as significant.

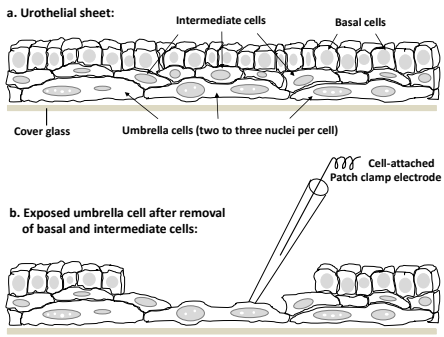

Figure 1 – Depiction of patch clamp of basal membrane of umbrella cell

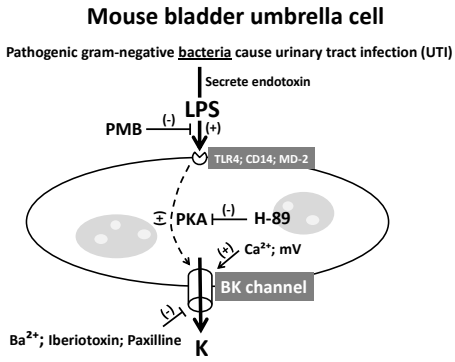

Figure 2 – Schematic of the LPS – BK signalling pathway in UTI pathogenesis

Results

We detected two K currents in the umbrella cells: a 28.3±1pS conductance current and a 200.6±4pS conductance current. 83% of patches exhibited the 28pS current channel, 52% patches displayed the 200pS current channel and 35% patches contained both the 28pS and 200pS current channels. With inside-out patch configuration in symmetrical condition where pipette [K] = bath [K], both currents disappeared at 0 mV holding potential which indicated they were K conductance channels. The 200pS K channel activity increased by elevating bath [Ca^{++}] with EC_{50} = 132 μM in inside-out patches. This channel's activity also showed voltage dependence. However, the 28pS K channel was insensitive to bath [Ca^{++}] changes. Iberiotoxin (3μM) and paxilline (5μM) specifically blocked the 200pS K channel outward and inward currents respectively which fit the BK channel's characteristics. RT-PCR and immunofluorescence further showed BK channel expression in the urothelium. To investigate the 200pS BK channel function in UTI, treatment of urothelial sheets with LPS (40 ng/ml) for 30 min significantly enhanced the BK channel activity in cell-attached patches measured by NPo from 0.50±0.13 to 1.62±0.31 ($p<0.001$). LPS did not change single BK channel conductance, but significantly increased the number of BK channels per patch from 1.09±0.09 to 2.83±0.31 ($p<0.001$) and single channel open probability from 0.28±0.08 to 0.70±0.09 ($p<0.001$). The LPS antagonist, polymyxin B (50ng/ml), abrogated the LPS effect measured in cell-attached patches. LPS receptors (TLR4, CD4 and MD-2) were also shown by RT-PCR in the urothelium, but not in the lamina propria or detrusor layers. The Downstream mechanism of LPS effect was also investigated by addition of H89 (10μM). H89 as a specific PKA inhibitor blocked the LPS effect on the BK channel activity in cell-attached patches, which

indicates PKA plays an important role in intracellular signalling thereby modulating umbrella cells response to bacterial LPS in UTI.

Interpretation of results

In this study, we demonstrate successful dissection urothelial sheets and identification of individual umbrella cells for patch clamp electrophysiology experiments. This technique is beneficial compared to enzymatic dissociation of urothelial cells, which generally resulted in loss of layer specificity. We also separated bladder wall into specific tissue compartments of urothelium, lamina propria and detrusor for molecular biologic studies. We identified a 200pS K channel on the basal membrane of the umbrella cell. This channel exhibited a BK channel phenotype due to: large conductance, calcium sensitivity, voltage gating, and blockage by iberiotoxin and paxilline. RT-PCR and immunofluorescence confirmed the presence of BK channel on the urothelium. LPS significantly up-regulated the BK channel activity and this effect was prevented by LPS antagonist (polymyxin B) and PKA inhibitor (H89). LPS receptor mRNAs were detected only in the urothelium, but not in the lamina propria or the detrusor, which accommodates the role of urothelium as the front barrier against bacteria invasion.

Conclusion

To our knowledge, this is the first time that BK currents have been reported in the basal membranes of umbrella cells. LPS significantly increased the BK channel activity through an intracellular PKA signalling pathway (Fig. 2), which represents an early host urothelial cell response to LPS. Targeting the BK channel regulatory mechanism has the potential to provide novel therapeutic approaches for UTI treatment besides antibiotics.

Disclosures

Funding: None **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Mouse **Ethics Committee:** Yale Institutional Animal Care and Use Committee