EPAC-RAP PATHWAY REGULATES BLADDER UROTHELIAL VESICLE EXOCYTOSIS

Hypothesis / aims of study
Bladder filling elicits vesicle exocytosis increasing the apical surface area of epithelial cells (measured by membrane capacitance). One stimulus governing apical exocytosis is cAMP. Although initially the effects of cAMP were solely attributed to activation of protein kinase A (PKA), recent findings uncovered a novel c-AMP sensor, Epac (exchange protein directly activated by cAMP) having two subunits Epac1 and Epac2. These molecules play a critical role in ion transport, vesicle trafficking and secretion, and barrier function via small GTPase Rap in various organs. Here we investigated the expression and role of Epacs in signaling apical membrane dynamics.

Study design, materials and methods
Bladders from female Sprague Dawley rats and New Zealand white rabbits were used for western blot and immunohistochemistry or for Rap activation assay. Transepithelial capacitance was measured by placing bladder mucosa in modified Ussing chambers and increasing hydrostatic pressure to mimic bladder filling (in the presence or the absence of Epac agonist: 8-pCPT-AM and Rap antagonist GGTI298, or non-selective Epac antagonist: ESI-09 or Epac 2 selective antagonist: ESI-05).

Results
Epac1 and Epac2 were expressed in the rat urothelium. Epac agonist (used in rabbit Urothelium) significantly increased transepithelial capacitance (30% vs 7% in control) in the absence of stretch; stretch-induced capacitance was reduced by ESI-09 (20% vs 80% in control) but not ESI-05. Rap was activated by Epac agonist and stretch stimulation. Rap antagonist had late inhibitory effect on increase of capacitance induced by Epac agonist.

Interpretation of results
Both Epac1 and Epac2 were expressed in the bladder epithelium, but the distribution and function was different. Epac1 plays an important role in augmenting bladder filling induced increases in urothelial surface area via Rap activation.

Concluding message
Urothelial Epac1 is a novel mediator in regulation of bladder capacity.

Disclosures
Funding: none Clinical Trial: No Subjects: ANIMAL Species: Rat, Rabbit Ethics Committee: The University of Pittsburgh Institutional Animal Care and Use Committee