Bradykinin Modulates Sensory Phenotype of the Human Urothelial Cell Line

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Introduction: Urothelial cells may crucially participate in the regulation of urinary bladder function. They display some sensory-neuron like properties, expressing sensory ion channels, such as transient potential vanilloid 1 channel (TRPV1) and releasing sensory mediators, including ATP and neurotrophins, which may affect the underlying afferent nerves. We have recently reported the presence of bradykinin B1 and B2 receptors on the human urothelial cell line (UROtsa) quantitatively corresponding to the expression pattern observed in a native human urothelial tissue (Ochodnicky et al., 2010). Here we explored bradykinin effects on UROtsa cell function including signal transduction mechanisms, urothelial mediator release and sensory ion channel/neurotrophic factor expression.

Methods: Activation of bradykinin signal transduction mechanisms was measured by fluo-4-AM calcium elevation assay and immunoblotting for intracellular calcium and ERK1/2 phosphorylation respectively. mRNA expression of TRPV1 and NGF in UROtsa was studied by real-time PCR. Extracellular release of ATP and NGF from UROtsa was measured by luciferin-luciferase assay and ELISA, respectively. Data are shown as means ± SEM of 3-4 experiments.

Results: Bradykinin concentration-dependently increased intracellular calcium concentrations (Emax= 4131±324 nM, EC50 = 5.6 nM). These elevations were abolished by selective B2 antagonist icatibant (1 µM), markedly inhibited by the phospholipase C inhibitor U73122 (3 µM), but unaffected by B1 antagonist R715 (1 µM). Although the calcium ionophore ionomycin (5 µM) invoked immediate ATP release from UROtsa cells, bradykinin did not have any effect. Bradykinin (100 nM) induced phosphorylation of the mitogen-activated kinase ERK1/2, which was inhibited by icatibant and by the protein kinase C inhibitor Gö6976 (1 µM). As this suggested a potential ability to regulate gene expression, we quantified bradykinin-induced alterations in mRNA expression of TRPV1 and nerve growth factor (NGF). A 24 h incubation with 100 nM bradykinin induced a 2-3 fold increase in both TRPV1 and NGF expression. NGF mRNA elevation was associated with a significant increase (up to 121±8 %) in constitutive NGF release from UROtsa to incubation medium.

Conclusion: We conclude that in the human urothelial UROtsa cell line, bradykinin acts on B2 receptors to elevate intracellular calcium and activate ERK1/2. While this does not lead to ATP release, it is associated with increased expression of TRPV1 and release of (NGF). These effects might be important in the regulation of afferent signaling in urinary bladder by the urothelium.

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Reference