Mediators of neurogenic inflammation in the urinary tract as key factors in the chronic pelvic pain syndrome/interstitial cystitis and bladder dysfunction

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ABSTRACT

Control of bladder function is one of the important processes in human body. Bladder pain syndrome (BPS)/ interstitial cystitis (IC) is a clinical syndrome of pelvic pain and either urgency or frequency without an identifiable cause, which is characterised by chronic inflammation. It is a debilitating condition, affecting both genders, but knowledge about its cellular basis is scant and treatment largely empirical.

In this thesis we undertook a comprehensive examination of contractility markers, tight junction proteins, and signalling molecules of human bladder, delineating the molecular differences between bladder regions and examining the changes in gene expression occurring during disease. We showed that NK1 and NK2 tachykinin receptors were significantly down-regulated in BPS patients. Tight junction proteins ZO-1, JAM-1 and occludin were similarly down-regulated, implicating increased urothelial permeability, whereas bradykinin B_1 receptor, cannabinoid receptor CB1 and muscarinic receptors M_3-M_5 were up-regulated. ASIC1b, ASIC2a, ASIC2b and ASIC3 were significantly up-regulated, whereas TRPV1 was down-regulated in BPS patients.

To elucidate the causative factors of the disease-induced remodelling of the bladder, we examined the microRNA profiles of BPS/IC patients, and identified 31 miRNAs differentially expressed in BPS patients, of which 28 were significantly up-regulated. Some of these miRNAs were predicted by bioinformatic tools to have tachykinin receptors or tight junction proteins as target genes. We showed that prolonged exposure of NK1R to its agonist Substance P caused a decrease of NK1R mRNA levels and a concomitant increase of regulatory microRNAs miR-449b and miR-500, which were also up-regulated in BPS patients. In a cell-based model we demonstrated a direct correlation between miR-449b, miR-500, miR-328 and miR-320 and a down-regulation of NK1R mRNA and/or protein levels.

Several microRNAs up-regulated in BPS/IC patients have the tight junction proteins as molecular targets. In order to understand the role of microRNA in epithelial integrity and tightness, we used a human ureteral cell line TEU-2, which can differentiate into a multilayer culture with tight junctions in its uppermost layer. Transfection of TEU-2 cells with miR-199a-5p, predicted to have TJ proteins claudin 1, JAM-1 or occludin as targets, precluded the formation of a tight epithelium. Our data indicate that MiR-199a-5p might affect the epithelial permeability by influencing the mRNA and protein levels of JAM-1

and claudin 1. Additionally, we showed that extracellular acidification below pH 6.0 or activation of protein kinase C decreased epithelial tightness of the differentiated TEU-2 epithelium.

In patients with bladder pain syndrome (BPS) the role of the bladder trigone is elusive, which has consequences for pain relief strategies during cystectomy. Our gene expression and organ bath muscle contractility experiments showed significant differences in expression profiles of the genes encoding contractile proteins, receptors and tight junction proteins between the bladder dome and trigone. These differences imply structural and sensory discrepancy between the two bladder regions, with implications for surgery and biopsy collection.

Our findings further the knowledge of the molecular mechanisms of BPS/IC, implicating several receptors, TJ proteins or channels in PBS pathophysiology. They highlight the complex processes of organ remodelling taking place in bladder dysfunction, and have relevance for other clinical conditions.