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INVOLVEMENT OF UROTHELIAL CRF RECEPTORS IN MODULATING BLADDER FUNCTION

Hypothesis / aims of study

The CRF signaling system is known to modulate pain responses and play a major role in the body's response to stress. While formally thought to act only at the level of the adenohypohysis, studies in humans support a role for CRF in visceral hypersensitivity and a potential role in the periphery. Recent evidence has shown that corticotrophin releasing peptides can exert direct effects within the pelvic viscera and are likely to influence stress-related visceral hypersensitivity. Corticotrophin peptides are involved in a number of functional pain syndromes and are now known to be expressed by peripheral tissues. In addition, visceral (and somatic) disorders including chronic pelvic pain/interstitial cystitis may also share common features including reports of stress as a contributing factor in exacerbating the symptoms. Feline interstitial cystitis (FIC), a chronic idiopathic cystitis in cats, exhibits similarities to humans diagnosed with bladder pain syndrome/IC. In both syndromes, alterations in urothelial (UT) signalling/barrier may contribute to the associated pathophysiology.

This study was undertaken to investigate: (1) urothelial cell expression and function of the corticotrophin releasing factor receptors (CRF1 and CRF 2) in rat bladder urothelial cells and feline urothelial cells from normal cats and cats with FIC (2) the trafficking events underlying transmitter (ATP) release by these cells and (3) the role of each of the receptor subtypes in urothelial signaling.

Study design, materials and methods

Urinary bladders were excised from female Sprague-Dawley rats (250-300 g), killed by inhalation of medical grade CO2 followed by thoracotomy and cardiac puncture. The bladders were cut open, gently stretched and pinned with the urothelial side up. Following an overnight incubation in MEM medium containing 2.5 mg/ml dispase, the urothelium was gently scraped from underlying tissue, treated with 0.25% trypsin and following resuspension cells were plated on collagen-coated cover slips.

Both healthy cats and cats diagnosed with interstitial cystitis (according to the NIDDK criteria) were housed in stainless steel cages and allowed to acclimatize to their environment for at least 3 months before study. Urinary bladders were removed from anesthetized (induction with 2% halothane and then maintained with α-chloralose 60–70 mg/kg) cats.After removal of tissue, animals were humanely sacrificed.

ATP release: Cat and rat urothelial cells grown for 2-3 on collagen–coated glass coverslips in a humidity-controlled incubator (5% CO_2; 37°C) were transferred into a perfusion chamber and superfused with an oxygenated Krebs solution (flow rate=0.5ml. min^{-1}), until a stable baseline level of ATP release was measured. Perfusate was collected (100 µl) at 30 s intervals following agonist stimulation and ATP levels quantified using a luciferin-luciferase reagent (ATP assay, Sigma Aldrich, St. Louis, MO). All data were normalized with respect to the maximum ATP released following application of ionomycin (5 µM) at the end of each experiment.

Live cell imaging of vesicular movement using membrane impermeant dyes

(FM): The fluorescent membrane impermeant FM dye, FM1-43 was used as an "activity marker" in order to track the movement of stimulus-evoked dye filled vesicles. Urothelial cells were incubated in HBSS solution containing the dye FM1-43 (5µM). In the absence of a stimulus, the plasma membrane will fluoresce but the inside of the cell will not. Membrane exocytosis is indicated by an overall increase in the fluorescence intensity with dye in the bath. Endocytosis is evidenced by the presence of dye containing vesicles in the cytoplasm

Interpretation of results

Our preliminary findings give functional evidence of expression of both receptor subtypes by bladder urothelial cells (both from cat and rat) in culture. The finding that the specific CRF2 antagonist evokes both trafficking and release responses in the absence of bath agonist points to the release of an endogenous ligand or ligands and suggests an potentially important role in UT cell physiology . The finding of a differential response to CRF between normal and FIC UT cells suggest that CRF receptor activity may be modified in FIC.

Concluding message

In animals, stress-sensitization is associated with induction of hyperalgesic states similar to many functional pain syndromes. It is likely that abnormalities in urothelial CRF receptor signaling may play a prominent role in bladder pain.

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What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee policies at each institution :Ohio State University, University of Pittsburgh.**