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Corticotrophin Releasing Hormone Regulates NLRP6 and Disrupts Mucosal Homeostasis in Functional Dyspepsia

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Background: Functional dyspepsia (FD) affects 15% of the population and is characterised by recurring upper gastrointestinal (GI) symptoms occurring in the absence of clinically identifiable pathology. The pathophysiology of FD is poorly understood, however recent work has demonstrated increased numbers of mucosal mast cells and eosinophils in patients with FD. Psychological stress is a key factor associated with the onset of FD and is a trigger of symptom exacerbations, however the biological mechanisms through which stress may lead to FD symptoms have not yet been defined. Recent pre-clinical work identified that psychological stress downregulates the innate epithelial immune protein NLRP6. NLRP6 has been shown to regulate GI mucus secretion and is responsible for activation of Interleukin 18 (IL-18). IL-18 is potently chemotactic for mast cells and eosinophils. We hypothesised that stress hormone levels are altered in patients with FD, leading to alterations in GI mucosal homeostasis and immune cell recruitment.

Methods: Goblet cell-like HT29-MTX-E12 (E12) cells were grown on permeable membranes over 21 days to form monolayers with apical-basolateral polarity. Cells were treated basolaterally with the stress hormone corticotrophin releasing hormone (CRH) (0.05μM or 0.5μM) for 16 hours. mRNA was extracted for qPCR analysis and immunoblots were performed on whole protein lysates. For mucous-layer analysis, monolayers were snap frozen and stained with alcian blue. FD patients were identified by the Rome III criteria; asymptomatic individuals were recruited as controls. Serum CRH levels were measured by ELISA. Pinch biopsies were collected from the duodenum of FD patients and non-FD controls. Biopsies were either formalin fixed and stained immunohistochemically or mRNA was extracted from enriched epithelial cell preparations for qPCR analysis.

Results: NLRP6 expression was increased in monolayers treated with 0.05μM CRH and decreased in monolayers treated with 0.5μM CRH ($n=3$, $p=0.02$), suggesting a dynamic regulation. CRH treatment at both 0.05μM and 0.5μM significantly increased mucus secretion in monolayers ($n=3$, $p=0.05$ and $p<0.0001$ respectively). Levels of CRH were significantly reduced in both duodenal tissue ($n=6$, $p=0.001$) and serum ($n=10$, $p=0.013$) of FD patients compared to non-FD controls. Duodenal NLRP6 was significantly increased in patients with FD compared to non-FD controls ($n=6$, $p=0.007$) and epithelial expression of NLRP6 was positively correlated with expression of IL-18 ($r=0.95$, $p=0.015$) in FD patients, but not non-FD controls.

Conclusion: This study has identified that CRH dynamically regulates NLRP6 *in vitro* and stimulates mucus secretion. Therefore, the concurrent decrease in CRH and increase in NLRP6 observed in FD patients provides a potential pathway through which psychological stress may lead to disruption of GI mucosal homeostasis and disease.