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Fecal Serine-Protease Activity: A Pathophysiological Marker and Possible Therapeutical Target in Diarrhea-Predominant Irritable Bowel Syndrome

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Digestive Disease Week®

and the

**107th Annual Meeting of the American
Gastroenterological Association Institute**

May 20-25, 2006, Los Angeles, CA

- Program of the Annual Meeting of the American Gastroenterological Association Institute, the American Association for the Study of Liver Diseases, the Gastroenterology Research Group, the Society for Surgery of the Alimentary Tract, and the American Society for Gastrointestinal Endoscopy
- Abstracts of Papers Accepted by the American Gastroenterological Association Institute
- Abstracts of Papers Accepted by the American Association for the Study of Liver Diseases
- Abstracts of Papers Accepted by the Society for Surgery of the Alimentary Tract

Targeting the VR1 receptor might be a therapeutic option in a large subgroup of patients with functional dyspepsia.

AAW-2006 - LOS ANGELES

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20.25 May 2005

Alternatively Activated Macrophages Contribute to Enteric Nematode Infection-Induced Intestinal Smooth Muscle Hypercontractility in Mice

Aiping Zhao, Robert M. Anthony, Justin E. Elfrey, Kathleen B. Madden, Rex Sun, William C. Gause, Joseph F. Urban, Terez Shea-Donohue

We showed previously that nematode infection induced an intestinal smooth muscle hypercontractility that depends on IL-13 activating STAT6. Macrophages orchestrate inflammatory responses and have been implicated in nematode infection. Depending on the cytokine environment, macrophages can differentiate into distinct subsets: classically activated macrophages (caMΦ) that are activated by IFN γ and are characterized by the secretion of nitric oxide via NOS-2; or alternatively activated macrophages (aaMΦ) that are induced by Th2 cytokines, IL-4 and IL-13, and are characterized by the upregulation of arginase-1. Aim: To determine the contribution of aaMΦ to *H. polygyrus* (*Hp*) infection-induced changes in murine intestinal smooth muscle function. Methods: BALB/c mice were infected with *Hp*, cured, re-infected (*Hp*²), and studied 14 days later. Groups of infected mice were injected with 0.2 ml of PBS- or clodronate-liposomes to deplete macrophages. Segments of jejunum from mice were suspended longitudinally in organ baths. After equilibration, the amplitude of the spontaneous contractions (SC) was measured over a 2-min period. Responses to acetylcholine (ACH), electric field stimulation (EFS), and 5-HT were determined. Real-time quantitative PCR was used to measure the mRNA expression of the molecular markers for caMΦ and aaMΦ from intestine. Results: *Hp*² infection increased F4/80 mRNA expression (4-fold), a macrophage molecular marker. In addition, infection upregulated the mRNA expressions for aaMΦ markers including arginase 1 (144-fold), macrophage mannose receptor (13-fold), MGL1 (5-fold), MGL2 (59-fold), and FIZZ1 (498-fold), but did not affect the expression of NOS-2, a caMΦ marker, indicating specific accumulation and activation of aaMΦ. *Hp*² infection induced an intestinal smooth muscle hypercontractility (Table), including elevated responses to ACH, EFS, and 5-HT, and increased amplitude of SC. However, depleting macrophages with clodronate prevented *Hp*²-infected mice from developing this hypercontractility. Conclusions: *Hp*² infection induces the recruitment and activation of aaMΦ that are required for the induction of intestinal smooth muscle hypercontractility. Thus, aaMΦ play a major role in IL-13-induced alterations in smooth muscle function that contribute to host defense against intestinal nematode infection.

	ACH (0.1mM)	EFS (20Hz)	5-HT (0.1mM)	SC
Control	15.7±1.0	8.2±1.4	6.4±1.4	4.7±1.7
<i>Hp</i> ² -PBS	36.8±4.9*	13.1±0.9*	13.7±1.7*	10.0±2.2*
<i>Hp</i> ² -clodronate	22.4±1.5 Φ	5.2±0.7 Φ	5.3±1.3 Φ	3.9±0.7 Φ

Values=Tensions in N/cm²; *p<0.05 vs Control; Φ p<0.05 vs *Hp*²-PBS; n \geq 4

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Timed Feeding Alters Clock Gene Expression in the Murine Stomach and Colon

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Background. Disruption of biological rhythms, such as occurs with shift work or time zone traveling, has been associated with gastrointestinal symptoms. These observations suggest a functional correlation between biological rhythms and gastrointestinal physiology. Biological rhythms are controlled by clock genes. A central clock is located in the suprachiasmatic nucleus of the brain. Clock genes have been identified in peripheral organs as well. Within a peripheral organ, clock genes can control the expression of a subset of tissue-specific genes, which may impact directly on its physiological function. Clock genes synchronize themselves to external time cues such as the light/dark cycle and feeding. We previously reported circadian expression of clock genes in the murine proximal colon. The aim of this study was to determine (1) whether circadian clock gene expression occurs in other parts of the gastrointestinal tract and if so, whether they are synchronized and (2) whether clock genes in the murine intestine can shift their expression patterns in response to a restricted daytime feeding schedule. Methods. Adult male C57BL/6j mice were kept under a 12 hr light/dark cycle (lights on at 7 AM). Group 1 was fed ad lib for one week. Group 2 and 3 were fed for the first 4 hours of the light cycle for 48 hrs and 1 week respectively. Total RNA was isolated from the stomach body, proximal- mid- and distal colon every 4 hrs starting at 4 AM (n=3 mice per time-point). A real-time multiplex PCR assay was developed for the detection of the clock genes period (PER2, PER3), cryptochrome (CRY1) and PAS helix-loop-helix transcription factor (Bmal1). PCR data were normalized against 18S. Statistical analysis was performed using a 2 way-ANOVA followed by a Tukey test. Results. Peak/trough ratios for PER2, PER3, CRY1 and Bmal1 in the proximal colon changed from 20.1, 23.3, 2.3 and 10 at baseline to 5.9, 3.2, 5.4 and 5.6 following 48 hrs of restricted feeding, respectively (P<0.05). Peak expression shifted from 8 PM to 4 PM, 8 PM to 8 AM, 4AM to 12 PM and 4 AM to 4 PM, respectively. This pattern persisted following 1 week of restricted feeding. Similar observations were made in the stomach body, mid- and distal colon. Conclusion. Restricted feeding can shift clock gene expression in the murine stomach and colon. The data suggest that the stomach and colon may have a time-keeping capability that allows for the physiological adaptation to environmental stimuli. We speculate that gastrointestinal symptoms may result from the inability of clock genes to make timely adjustments to changes in environmental cues, such as occurs with time zone traveling and shift-work.

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107th - AGA - 2006. (LA)

Fecal Serine-Protease Activity: A Pathophysiological Marker and Possible Therapeutic Target in Diarrhea-Predominant Irritable Bowel Syndrome

Richard Roka, Andras Rosztoczy, Mathilde Leveque, Ferenc Nagy, Janos Lonovics, Rafael Garcia-Villar, Jean Fioramonti, Tibor Wittmann, Lionel Bueno

Background: The pathogenesis of irritable bowel syndrome (IBS) remains only partially understood, and no specific or universally effective patient management procedure has been developed so far. Activation of receptors for proteases located on colonocytes may participate in immune mucosal stimulation. Consequently, our study was designed to evaluate if colonic luminal serine-proteases may be altered in IBS. Methods: Fecal samples of 38 IBS patients (IBS-D:17; IBS-C:14; IBS-A:7), 15 patients with ulcerative colitis (UC) and 15 healthy controls were studied. Fecal serine-protease activity was determined photometrically using azocasein as a proteolytic substrate; fecal pancreatic elastase-1 and mast cell tryptase content were measured by ELISA. Fecal secretory leukocyte protease inhibitor (SLPI) concentration was determined by ELISA in control subjects and in patients with diarrhea-predominant IBS. Results: Fecal serine-protease activity was 3-fold higher (p<0.001) in patients with diarrhea-predominant IBS (67.9±10.8 U/mg protein), than in both controls (16.4±5.0 U/mg protein) and IBS patients with either constipation (16.0±7.0 U/mg protein) or alternating bowel habits (16.4±7.1 U/mg protein). Fecal serine-protease activity was not correlated with the frequency of bowel movements in all groups. Elevated serine-protease activity was also detected in stools of UC patients (67.4±15.3 U/mg protein). No significant difference was observed in fecal mast cell tryptase and pancreatic elastase concentrations between all groups, and in fecal SLPI concentration between controls and diarrhea-predominant IBS patients. Conclusions: Fecal serine-protease activity is markedly elevated in patients with diarrhea-predominant IBS. This increase, however, is not coupled with changes in either mast cell tryptase or pancreatic elastase concentrations, and may participate in the mucosal immune stimulation described in such patients and subsequently in the genesis of their symptoms.

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Inhibition of the Putative Anion Transporter (PAT-1, SLC26A6) Impairs the Ability of Upper Villous Epithelial Cells to Regulate Intracellular pH During H⁺-Dipeptide Transport

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Carrier-mediated transport of di- and tripeptides via the small intestinal oligopeptide transporter PEPT1 accounts for the majority of dietary amino acids absorbed after protein digestion. Proton influx during PEPT1 di-/tripeptide uptake by intestinal epithelium requires maintenance of intracellular pH (pH_i) to sustain the driving force for peptide absorption. Studies performed in the absence of CO₂/HCO₃⁻ in the medium have demonstrated that the apical membrane Na⁺/H⁺ exchangers NHE3 and NHE2 can effectively minimize pH_i acidification during proton di-/tripeptide transport. However, the contributions of acid-base transport proteins in the regulation of pH_i during H⁺-peptide transport have not been fully investigated under physiological conditions (i.e., in the presence of CO₂/HCO₃⁻). Therefore, we used BCECF microfluorimetry to measure pH_i and to investigate the role of acid-base transporters during exposure of the epithelium to the dipeptide glycyl-sarcosine (gly-sar) in Krebs' HCO₃⁻ Ringers. Measurements were made in the upper villous epithelium of the murine duodenum because this is a major site of PEPT1 activity. Surprisingly, we found that inhibition of luminal Cl⁻/HCO₃⁻ exchange with either 100 μM niflumic acid or application of bilateral Cl⁻ free conditions resulted in a greater proton influx during gly-sar exposure (NFA = -7.05±0.89; Cl⁻ free = -15.93 ± 0.21 mM/min; control = -0.83 ± 0.94 mM/min; n = 3; p<0.05). Since PAT-1 is the major apical membrane Cl⁻/HCO₃⁻ exchanger in the upper duodenal villous epithelium, we measured the effect of gly-sar transport on pH_i in wild-type (WT) and PAT-1 knockout (KO) duodena. Exposure to gly-sar resulted in a greater proton influx in the PAT-1 KO as compared to WT (-9.54 ± 3.40 vs. -0.07 ± 0.79 mM/min, n = 3, p<0.05). The effect of gly-sar on pH_i was also compared between WT and carbonic anhydrase II (CAII) knockout intestine because previous studies have shown that CAII interacts with PAT-1 and plays a role in facilitating peptide transport in physiological solutions. Gly-sar-induced acidification was significantly greater in the CAII KO intestine (-14.37 ± 1.71 vs. -1.68 ± 1.03 mM/min, n = 4, p<0.05). We conclude that PAT-1 Cl⁻/HCO₃⁻ exchanger in conjunction with CAII contributes to protection against intracellular acidification during H⁺-dipeptide absorption in the upper villous epithelium, perhaps by operating in Cl⁻_{out}/HCO₃⁻_{in} mode as a base-loading mechanism for the epithelial cell. Supported by NIH.

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Glucose-Dependent Insulinotropic Polypeptide (GIP) Stimulates Transepithelial Glucose Transport in Mouse Jejunum *In Vitro*

Selvi Krishnan, M. Michael Wolfe, John H. Schwartz, Satish K. Singh

Background: Recent reports have suggested a key role for GIP, a regulatory peptide synthesized by intestinal enteroendocrine K-cells, in the pathogenesis of obesity. Previous studies from our group have demonstrated effects of GIP on nutrient deposition. Thus, we hypothesized that GIP may regulate intestinal glucose transport directly and thereby influence systemic glucose homeostasis. Objective: To characterize the effects of GIP on jejunal glucose transport *in vitro*. Methods: Stripped proximal jejunal epithelium from fasted mice was mounted in Ussing chambers. The serosal side was bathed with a bicarbonate-Ringer solution containing 5mM glucose, and the mucosal side, with a solution containing 10mM 3-O-methyl glucose (3OMG), a non-metabolizable glucose analog. Mucosa-to-serosa fluxes of 3OMG (Jms3OMG) and short circuit current (Isc) were determined in the presence and absence of 1μM and 10nM GIP, with and without the inhibitors of glucose transport, phlorizin (20μM) and phloretin (250μM). To examine whether GIP stimulates epithelial cells directly, viable enterocytes isolated by Ca²⁺-chelation were incubated with GIP for 4hr after which cAMP was measured by RIA. Results: GIP increased both Isc and Jms3OMG significantly. The increase in Jms3OMG was greater than the change in Isc (68% vs. 53%). As such, the GIP-induced increase in Jms3OMG was primarily electrogenic, with a small electroneutral