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Role of Nesfatin-1, the Novel Satiety Hormone, in the Mechanism of Gastric Mucosal Defense. Importance of Endogenous Prostaglandins and Sensory Afferent Nerves

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Nesfatin-1 derived from the nucloebindin2 (NUCB2) precursor is a novel 82 amino acid polypeptide recently detected in the rodent stomach. Nesfatin-1 inhibits nocturnal food intake and body weight gain but its role in the mechanism of gastric mucosal defense remains unknown. We determined the effect of intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) administration of nesfatin-1 against gastric mucosal lesions induced by application of 75% ethanol (1.5 ml i.g.) and water immersion restraint stress (WRS) (series A) without or with co-treatment with 1) the non-selective (indomethacin; 5 mg/kg i.p.) or selective COX-1 (SC-560, 5 mg/kg i.p.) and COX-2 inhibitors (rofecoxib 10 mg/kg i.g.), and 2) vagotomy due to cutting of vagal nerves and 3) blockade of sensory nerves by large dose of capsaicin (125 mg/kg s.c.) or 4) inhibition of vanilloid receptor (VR-1) by capsazepine (10 mg/kg i.g.). The area and number of gastric lesions was measured by planimetry, the gastric blood flow (GBF) determined by H_2-gas clearance technique, PGE_2 generation in gastric mucosa and plasma nesfatin levels were determined by specific RIA and NUCB2-, COX-1-, COX-2-, IL-1β-, and TNF-α mRNAs and proteins were assessed RT-PCR and Western Blot. Ethanol and WRS induced mucosal hemorrhagic lesions without affecting NUCB2mRNA expression and plasma nesfatin-1 level. Nesfatin-1 (0.1-30 μg/kg i.p.) or (25-1800 ng/rat i.c.v.) dose-dependently attenuated ethanol- and WRS-induced gastric lesions while producing a significant increase in GBF, plasma nesfatin-1 level and mucosal RIA-PGE_2 generation. The protective effects of nesfatin-1 were significantly attenuated by indomethacin and rofecoxib but not altered by SC-560. Co-administration of 16,16 dm PGE_2 (5 μg/kg i.p.) with nesfatin-1, restored the protective activities of this peptide in rats treated with indomethacin, SC-560 and rofecoxib. Vagotomy, capsaicin denervation or capsazepine also significantly reduced the gastroprotective activity of nesfatin-1 and the addition of exogenous CGRP (10 μg/kg s.c.) restored the protection and mucosal hyperemia of this peptide in capsaicin-denervated rats. COX-2 was upregulated in injured gastric mucosa and this effect were further enhanced in nefatin-1 treated rats. The IL-1β and TNF-α expression and plasma levels of these cytokines were significantly elevated in ethanol- and WRS exposed rats and these effects were significantly attenuated by nesfatin-1. We conclude that nesfatin-1 exerts gastroprotective activity *via* mechanism involving activation of PG-COX-2 system, activation of vagal and sensory nerves releasing CGRP and suppression of the proinflammatory cytokines.

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Netrin Biosynthesized By Enteric Neurons Mediates the Attraction of Vagal Sensory Axons to the Fetal Gut

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Vagal sensory axons innervate the gut during fetal life. By applying DiI to nodose ganglia at a range of gestational ages in mice, we have found that vagal sensory axons descend to the stomach and small bowel by E12 and E14 respectively, following the rostral-caudal migration of crest-derived cells. DiI-labeling has furthermore revealed that vagal sensory axons fail to innervate the aganglionic gut of mice lacking the tyrosine kinase receptor, Ret. Previous work has shown that netrin is expressed in the bowel wall and, by acting on its receptor Deleted in Colorectal Cancer (DCC), mediates the guidance of vagal sensory axons to the developing gut. We therefore tested the hypothesis that developing enteric ganglia express netrin. Crest-derived and non-crest-derived cells of E15 rat gut were separated by positive and negative immunoselection with antibodies to $p75^{NTR}$, a marker for crest-derived cells in the fetal bowel. The $p75^{NTR}$-immunoreactive cells were then cultured for 6 days in media supplemented with the Ret ligand, glial cell derived neurotrophic factor (10 ng/ml) to promote neuronal differentiation. Transcripts encoding netrins-1 and -3 were found by RT-PCR in the cultured enteric neurons and in the freshly immunoselected non-crest-derived cells, but not in the non-cultured immunoselected crest-derived precursors. To test whether enteric neurons take up, as well as synthesize netrin, the $p75^{NTR}$-immunoreactive cells were cultured for 6 days in 3-dimensional collagen gels with stably transfected 293-EBNA cells expressing c-Myc-tagged netrin-1. Controls included crest-derived cells cultured alone or with parental 293-EBNA cells. In all 3 types of culture, neurons (identified by their expression of PGP9.5) were found to be netrin-immunoreactive. Cultures were immunostained with antibodies to c-Myc to identify netrin secreted by the transfected 293-EBNA cells. Although the netrin-1-expressing 293-EBNA cells were c-Myc-immunoreactive, the neurons that developed from the crest-derived cells were not. Neurons thus express but do not take up netrin. To further investigate the role of netrin synthesized by enteric ganglia, as opposed to from the enteric mesenchyme, protein was extracted from the bowel of E14 Ret -/-, Ret +/- and Ret +/+ fetal mice. Quantitative analysis of netrin-1 identified through Western blotting revealed no significant difference in total amount of netrin-1 protein per gut. We propose that the neuronal origin of the netrin gradient plays a more important role than the aggregate amount of enteric netrin in guiding vagal axons to their correction locations in the developing bowel. Supported by AGA, CIHR, NS 15547/12969.

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Enteric Nitrergic Neuron Defect and Gut Dysfunction in a Rat Model of Parkinson's Disease

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Parkinson's disease (PD) is characterized by centrally-related motor and non-motor symptoms mainly involving the gastrointestinal (GI) tract (e.g. constipation). The latter disturbances suggest an involvement of the innervation supplying the digestive system. Typical cytoplasmic inclusions (Lewy bodies) have been shown in the enteric nervous system (ENS) of patients with PD. Aim: this study was designed to establish functional and neurochemical changes affecting the GI tract in a model of PD based on the stereotaxic infusion of the neurotoxin 6-hydroxydopamine (6-OHDA) into the rat nigrostriatal tract. Methods: rats received two injections of 6-OHDA (3 μg/3 μL) or saline (controls) in the right medial forebrain bundle, in order to induce a relevant lesion of the dopaminergic pathways. Control and 6-OHDA treated rats were monitored for 4 weeks, during which daily fecal output was assessed. After 4 weeks, 6-OHDA and control animals were sacrificed. Brains were collected to verify the lesion of dopaminergic cell bodies and terminals. A 10 cm-long segment of distal colon was used for induction of propulsive activity triggered by intraluminal infusion of Tyrode solution. Whole-mounts of ileum, proximal and distal colon were processed for double-labeling immunohistochemical analysis using different neurochemical markers (HuC/D, nNOS, VIP and pChAT). Results: the daily fecal output was reduced by 50% in 6-OHDA-treated rats. In isolated colonic segments, the efficiency of peristalsis was reduced in 6-OHDA rats vs. controls. In particular, the residual volume in 6-OHDA-treated rats was enhanced by 40%. The numbers of total HuC/D-labeled myenteric neurons (calculated as ratio of the total area) showed no difference between treated vs. controls in the ileum (307.1±29.3 vs. 307.9±30.9), proximal (435.3±23.4 vs. 435.4±27.8) and distal (398.8±12.7 vs. 390.6±46.5; n=4) colon, respectively. Conversely, the percentage of nNOS myenteric neurons in 6-OHDA-treated rats decreased in the ileum (-21.7% P<0.0001; n=4) and proximal colon (-15.5% P<0.002; n=4), but not in the distal colon. VIP- and ChAT-containing myenteric neurons of the ileum, proximal and distal colon did not change in 6-OHDA vs. controls. Conclusions: A selective nigrostriatal dopaminergic denervation is associated with selective nitrergic deficiency in the ENS and with functional GI abnormalities (i.e. constipation and reduced peristaltic efficiency). This study provides a basis to understand the brain-to-enteric neural system cross-talk in the pathophysiology of gut dysfunction in patients with PD.

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Detection of Small Intestinal Bacterial Overgrowth in Irritable Bowel Syndrome: Comparison of Lactulose Hydrogen Breath Test with Scintigraphic Oro-Cecal Transit Study

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Aims: The lactulose hydrogen breath test (LHBT) has been employed to diagnose small intestinal bacterial overgrowth (SIBO), with recent reports in the US suggesting up to 80% of IBS patients have SIBO. We hypothesized that the interpretation of the LHBT has been misleading and reflects transit time in the small intestine as opposed to bacterial overgrowth. To test our hypothesis, IBS patients underwent combined LHBT and scintigraphic oro-cecal transit study (SOCTS) to determine whether the rise in hydrogen in the LHBT occurred before or after the Tc99 in the test meal had reached the cecum. Methods: Rome II IBS participants were recruited prospectively from an outpatient gastroenterology service with a referral population >500,000 in Ontario. After an overnight fast (>10h), participants provided a baseline hydrogen breath sample. Patients then ingested a test meal of 15ml Duphalac (10 g of lactulose) and 20MBq 99m-Tc-sulphur colloid in 100 ml water and were placed recumbent under a GE XRT gamma camera. Abdominal images were recorded every 10 minutes for 3 hours and simultaneously, breath samples were collected to be analyzed for hydrogen level. The test results were interpreted by independent observers. Using the criteria employed in clinical studies, the LHBT was determined to be abnormal if the hydrogen level rose greater than 20 ppm ≥ baseline within 3 hours. The test meal was determined to be in the cecum when there was a significant increase in cecal radioactivity as determined by decay corrected geometric mean counts using anterior and posterior projections. Results: 26 Rome II IBS participants have been enrolled in the study with 13 completions, 8 awaiting complete analysis of test results, and 5 drop outs. Of the 13 patients who have completed the study (12 female, 1 male), 9 were diarrhea predominant, 2 were alternating, and 2 were constipation predominant. 9/13 (69%) participants met criteria for an abnormal LHBT. Of these participants, 7 had diarrhea predominant symptoms, 1 alternating, and 1 had constipation predominant symptoms. In 8/9, the Tc99 reached the cecum before their LHBT results became abnormal. The median time for the test meal to reach the cecum in these patients was 40 minutes (range 10-70 minutes) while the median time for an abnormal LHBT was 65 minutes (range 40-150 minutes). 1/9 (11%) participants had an abnormal LHBT result before their test meal reached the cecum. This patient's test meal reached the cecum in 60 minutes with an abnormal LHBT at 40 minutes. Conclusions: These results suggest that most of the abnormal LHBT's in patients with IBS can be explained by transit to the cecum as opposed to SIBO.

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Gastrointestinal Permeability (GIPerm) Is Increased in Family Members of Children with Functional Abdominal Pain (FAP) and Irritable Bowel Syndrome (IBS)

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Background: Increased GIPerm has been described in children with FAP/IBS and adults with IBS. We sought to determine if baseline GIPerm is increased and if ibuprofen induces a greater increase in GIPerm in parents and siblings of children with FAP/IBS vs. Control families without children with FAP/IBS. **Design/Methods:** Site specific GIPerm testing was carried out in two groups of families: 1) parents and siblings of children previously identified as having FAP or IBS and 2) parents and their children with no history of FAP or IBS in the children (Controls). Parents and children ingested a solution containing sucrose (10 g/dL), lactulose (5 g/dL), mannitol (1 g/dL), and sucralose (1 g/dL) after a 4 hr fast following the evening meal. Urine was collected for 24 hr. Percent recovery of the sugars and sugar ratios were calculated: 1) from the time of ingestion through the first morning urine (overnight), 2) after the first morning urine through 24 hr after ingestion (day/evening), 3) and for the entire 24 hr period. In the adults the GIPerm test was repeated following the ingestion of 400 mg of ibuprofen every 8 hr for 3 doses. Data were log transformed for analysis. **Results:** n=23 FAP/IBS families (14 fathers, 22 mothers, 21 children) and n=14 Control families (7 fathers, 14 mothers, 18 children). In children, controlling for age, sex, and family, overnight gastric (% sucrose recovery) and gastric/proximal bowel (sucrose/lactulose ratio) GIPerm were greater in siblings of FAP/IBS children than in Controls (Gastric: 0.08 ± 1.2 vs. 0.02 ± 1.2, P<0.0005; Proximal: 0.28 ± 1.22 vs. 0.11 ± 1.42, respectively, P=0.03; mean ± SE).