biopsies. Based on histological and serological assays patients were categorized as HP+ or HP-. Leptin levels in serum and biopsies, and IL1-β levels in biopsies, were determined by specific ELISAs. Gastric biopsies were graded by type of gastritis and by level of gastritis according to the updated Sydney classification. Results: The 129 HP+ and 107 HP- subjects did not differ in mean age (65 ± 12 years). Normal histology was present in 16 (7.0%) individuals, chronic gastritis (CG) was present in 109 (46%), while atrophy or intestinal metaplasia (A/IM) was identified in 111 (47%). As expected, gastritis score was higher in the HP+ compared to the HP- group (mean 2.7 ± 1.6 vs. 1.8 ± 1.7, p<0.001). Similarly, the prevalence of A/IM was higher in the HP+ compared the HP- group (62% vs. 29%, p<0.001). HP+ individuals had lower levels of plasma leptin (median 2151 pg/ml IQR (869-4138) vs. 4640 (1685-7273), p=0.009) and integrated (mean antral and fundic) gastric leptin (median 103 pg/mg protein IQR (67-182) vs. 129 (87-190), p=0.048) compared to HP- patients. Among all subjects plasma leptin was lowest in individuals with normal histology, followed by those with A/IM, and highest in those with CG (median 2058 pg/ml, IQR (465-4640), 2151 (891-4741), and 3624 (1381-6971), respectively; p=0.021). Among HP+ individuals, there was a non-significant trend towards lower levels of antral leptin in the setting of A/IM compared to CG; median 71 pg/mg protein IQR (33-160) vs. 118 (42-186). Gastritis score correlated with plasma leptin in the HP+ subset (r=0.20, p=0.033) but not in the HP- group; similarly, antral IL1-β correlated with antral leptin in the HP+ subset (r=0.41, p<0.001), but in not the HP- group. Conclusions: HP colonization is associated with reduced levels of gastric and circulating leptin. Gastric atrophy is associated with lower levels of circulating leptin compared to chronic gastritis. Gastric inflammation in the setting of HP colonization may play a role in nutrient absorption and energy metabolism through its effect on gastric and circulating leptin.

M1669

Active and Inactive Pools of nNOS in the Nerve Terminals in Mice Gut: Implications for Nitrergic Neurotransmission

Y. M. Rao, Arun Chaudhury, Raj K. Goyal

The regulation of nitrergic neurotransmission is not well understood. We have previously reported the presence of calmodulin (CaM) and ser847 phosphorylated nNOS in the nerve terminals. We have now extended those studies to document active and inactive pools of nNOS in the nerve terminals. Mice gut varicosities were obtained by ultracentrifugation/sucrose gradient purification and confirmed as nitrergic by colocalization of nNOS with synaptophysin. Immunoprecipitation with CaM and subsequent probing with nNOS1422-1433, or immunoprecipitation with nNOS1422-1433 and probing with CaM, confirmed that only a fraction of nNOSα dimer was bound with CaM. In additional experiments, calmodulin-lacking nNOS, the supernatant after CaM-immunoprecipitation of the varicosity extracts was obtained and concentrated. The CaM-bound and CaM-lacking fractions were electrophoresed and hybridized with anti-serine847-phospho-nNOS antibody (Santa Cruz). We found that CaM-bound nNOSα dimer did not react with ser847-phospho-nNOS. On the other hand, a portion of nNOSα dimer, nNOSβ dimer and nNOSα monomer that lacked bound-CaM reacted with ser847-phospho-nNOS antibody. *In Vitro* assays of NO production revealed that only the CaM-bound dimeric nNOSα was catalytically active; all other forms were inactive. These results suggest that: 1) at any given time, only a fraction of nNOSα dimers is CaM-bound and is catalytically active. A larger pool of nNOS in the nerve terminals is phosphorylated at ser847 and is catalytically inactive. 2) The amount of catalytically active pools may be regulated by serine847 phosphorylation and equilibrium between dimers and monomers of nNOSα. These studies provide a mechanism for the regulation of nitrergic neurotransmission during de novo synthesis of nitric oxide by nNOS.

M1670

Choline Transporter: A New Label for Cholinergic Nerves in the Human Enteric Nervous System That Reveals Cholinergic Nerves in Colonic Mucosa

Andrea M. Harrington, Margaret Lee, Sim-Yee Ong, Pam Farmer, John M. Hutson, Bridget R. Southwell

Acetylcholine is a major neurotransmitter in the intestine. Cholinergic neurons are labelled using antibodies against choline acetyltransferase (ChAT) and vesicular acetylcholine transporter (VAChT). Choline uptake into nerve terminals is essential in the synthesis of acetylcholine, is the rate limiting step and occurs via the high affinity choline transporter (CHT) [1]. CHT-immunoreactivity is present in neurons containing VAChT and ChAT in the human central nervous system [2] and the rat enteric nervous system [3]. This study examined if the CHT antibody labels cholinergic nerve fibers in human intestine. METHODS: Human ileum and colon biopsies (n=4) were fixed, frozen, sectioned and processed for fluorescence immunohistochemistry. Double labeling was performed using antibodies against CHT, synaptophysin, cChAT, VAChT, nitric oxide synthase (NOS), substance P (SP), vasoactive intestinal peptide (VIP) and c-Kit to label interstitial cells of Cajal. RESULTS: CHT-immunoreactivity was present in many nerve fibers in circular and longitudinal muscle, myenteric and submucosal ganglia, submucosa and mucosa. CHT completely colocalised with VAChT and cChAT. In myenteric ganglia and circular muscle, there was some colocalisation with SP, but little with VIP or NOS in nerve fibers. Interstitial cells of Cajal were closely intertwined with nerves containing CHT-immunoreactivity in the circular muscle. Although cholinergic nerves are known to be present in colon mucosa, VAChT does not label these fibers in human colon. CHT-immunoreactivity was prominent in nerve fibers in the colon mucosa and submucosa. CHT was present in nearly all VIP and some SP nerve fibers in the mucosa. CONCLUSIONS: This study shows CHT antibodies labelled cholinergic enteric nerves in human ileum and colon and suggests it is a useful additional cholinergic marker. Importantly, CHT antibody labels nerve fibers in the mucosa that are not labeled by VAChT, providing the first visualization of these known cholinergic nerves and a label for these previously invisible nerve fibers. REFERENCES 1. Okuda, T. and T. Haga, High-affinity choline transporter. Neurochem Res, 2003. 28(3-4): p. 483-8. 2. Kobayashi, Y., et al., Distribution of the high-affinity choline transporter in the human and macaque monkey spinal cord. Neurosci Lett, 2002. 317(1): p. 25-8. 3. Harrington, A.M., J.M. Hutson, and B.R. Southwell, High affinity choline transporter immunoreactivity in rat ileum myenteric nerves. Cell Tissue Res, 2007. 327(3): p. 421-431.

M1671

Modulation of Brainstem Vagal Inhibitory Circuits in Response to Application of CRF and Oxytocin. *In Vitro* Studies

Kirsteen N. Browning, R. Alberto Travagli

Patients with functional dyspepsia (FD) have symptoms such as impaired gastric emptying, reduced stomach compliance and early satiety that are aggravated by stressful events. The pathophysiological mechanisms are incompletely understood, although several lines of evidence point toward an impairment of vagal sensory-motor circuits. We have shown that inhibitory vagal brainstem circuits undergo dramatic short-term plastic changes; we hypothesize that derangement, or untimely deviations, of this plasticity may exacerbate FD symptoms. The aim of the present study was to determine whether corticotropin releasing factor (CRF), one of the leading mediators of stress-related alterations in gastrointestinal functions, induces plasticity within inhibitory vagal pathways. We used oxytocin (OXY), as a model peptide to test its effects on brainstem GABAergic synapses, since it is well known that OXY, released following a meal, modulates excitatory vagal circuits. Using whole cell patch clamp recordings from the rat brainstem were used to show that CRF increased inhibitory GABAergic synaptic transmission to a subpopulation of gastric-projecting DMV neurons. In fact, CRF increased the amplitude of evoked inhibitory postsynaptic current (eIPSC) in 5 of 8 neurons; the ability of CRF to increase the frequency, but not amplitude, of miniature (m)IPSCs in a further 3 of 6 neurons suggests these effects occured via actions at presynaptic receptors. In naïve brainstem slices, OXY had no effect on inhibitory synaptic transmission; OXY neither inhibited the amplitude (n=10) nor the frequency of mIPSCs (n=6). Following exposure to CRF (and recovery from its actions), re-application of OXY inhibited eIPSC amplitude and decreased the frequency of mIPSC in all neurons tested. Again, the ability of OXY reapplication to inhibit mIPSC frequency but not amplitude suggests the involvement of presynaptic receptors. Immunohistochemical analysis on DMV neurons characterized electrophysiologically showed that oxytocin-1 receptors are present on GABAergic terminals of naïve rats only after CRF pretreatment. These results suggest that CRF induced plasticity changes vagal inhibitory circuits that uncover the ability of oxytocin to modulate GABAergic currents. While adaptive plastic responses are essential to adjust to ever-changing physiological conditions, untimely deviations of this plasticity may have pathophysiological consequences such as exacerbation of stress-induced FD symptoms. Supported by DK 55530

M1672

Differential Effect of CB1 Receptors On the Discharge of Afferent and Efferent Fibres Supplying the Rat Jejunum

Jemma Shaw, Wen Jiang, David C. Bulmer, David Grundy

Background and aims: Cannabinoid (CB_1) receptors are expressed on GI sensory neurons and are suggested to play a role in the regulation of food intake and nociception (Gomez *et al* 2002, Agarwal *et al* 2007). Expression of CB_1 in the nodose is influenced by nutritional status (Burdyga *et al* 2004). Our aim was to determine the functional response of afferent and efferent fibres supplying the proximal jejunum to the CB_1 agonist docosatetraenylethanolamide (DEA) in fed and fasted animals. **Methods:** Experiments were performed in anaesthetised Wister rats (250-350g), either fed ad libitum or fasted for 24hrs, with free access to water. The jugular vein, carotid artery and trachea were canulated, and a midline laparotomy performed to allow intraluminal pressure recording from the proximal jejunum. A single paravascular nerve bundle was isolated from a mesenteric arcade and attached to an electrode for recording either afferent or efferent impulse traffic. Drugs were applied intravenously. Analysis included paired Student's t test and data is presented as Mean ± SEM. **Results:** DEA (1mg/kg) had a depressor effect on blood pressure (pre 110.1 ± 3.0 mmHg, post 96.6 ± 4.7 mmHg P=0.002 n=14) but surprisingly little effect on afferent nerve activity in fed animals (pre 23.4 ± 4.6 imp/s, post 22.4 ± 4.3 imp/s n=7). In fasted animals the afferent response to DEA was augmented (pre 35.2 ± 10.1 imp/s, post 39.9 ± 11.5 imp/s P=0.04 n=5), however the blood pressure effect was attenuated (pre 105.1 ± 2.9 mmHg, post 97.7 ± 5.9 mmHg n=10). In contrast, DEA (1mg/kg) caused a significant and prolonged increase in efferent firing (pre 105.3 ± 5.3 imp/s, post 145.7 ± 17.9 imp/s, P<0.0001 n=9) which was diminished in fasted animals (P<0.0001 by 2way ANOVA compared to fed n=5). The effect of DEA on efferent firing was dose dependent, at 0.3mg/kg the response rapidly recovered to baseline (peak 22.5 ± 4.6 imp/s n=9) and was reproducible on repeat administration allowing evaluation of the sequential effect of vagotomy and ganglionic blockade. Bilateral cervical vagotomy had no effect on the DEA response (peak 22.8 ± 3.2 imp/s n=5), however after hexamethonium (10mg/kg) the DEA response was reversed (peak -5.9 ± 2.4 imp/s P<0.0001 n=5). **Conclusions:** DEA has a modest on intestinal afferent firing but a profound effect on efferent function which may be modulated by changes in nutritional status. The persistent response after vagotomy and block by hexamethonium suggests DEA is acting centrally, although there may be an inhibitory effect at the level of the sympathetic ganglia.

M1673

Peripheral Injection of Ghrelin Induces FOS Expression in the Dorsomedial Hypothalamic Nucleus in Rats

Miriam Goebel, Anna-Sophia Wisser, Peter Kobelt, Andreas Stengel, Ivo R. van der Voort, Rüdiger W. Veh, Bertram Wiedenmann, Burghard F. Klapp, Yvette Tache, Hubert Monnikes

Peripheral ghrelin has been shown to act as a gut-brain peptide exerting a potent orexigenic effect on food intake. The dorsomedial nucleus of the hypothalamus (DMH) is innervated by projections from other nuclei being part of the network of brain areas controlling energy homeostasis, among others NPY/AgRP-positive fibers arising from the arcuate nucleus (ARC). The **aim** of the study was to determine if peripherally administered ghrelin would affect neuronal activity in the DMH, as assessed by Fos expression. **Methods:** The study was performed in non-fasted Sprague-Dawley rats. At the end of the dark phase animals received intraperitoneally (IP) 3 nmol ghrelin (n=6) or vehicle (0.15 M NaCl; n=6). Brains were removed 90 min after injection. Fos immunohistology and additional double staining with