● G3144

NEURONAL AND ENDOTHELIAL NITRIC OXIDE SYNTHASES WERE NOT DETECTED IN C-KIT-POSITIVE INTERSTITIAL CELLS OF CAJAL IN ADULT HUMAN STOMACH AND INTESTINE. C. He, S.M. Miller, M.G. Sarr*, J.H. Pemberton*, G. Farrugia and J.H. Szurszewski. Dept. of Physiology & Biophysics and Div. of Gastroenterology and Dept. of Surgery, Mayo Clinic, Rochester, MN 55905.

Interstitial cells of Cajal (ICC) may function as pacemaker cells of the gut. How they interact with smooth muscle cells and intrinsic nerves to mediate contractions of the gut is not completely understood. ICC in dog colon have been reported to contain nitric oxide synthase (NOS), the enzyme producing nitric oxide (NO), an important inhibitory mediator in the gut. It has also been suggested that ICC and intrinsic nerves containing NOS in the gut might interact with each other to modulate gut motility. Few, if any studies, have examined whether ICC in the adult human gut contain NOS or whether they are innervated by NOS-containing nerves. Pieces of normal human stomach (n=10), jejunum (n=12) and large intestine (sigmoid colon, n=3) were obtained as surgical waste material, fixed under standard conditions using 4% paraformaldehyde or 4% paraformaldehyde containing picric acid, cryoprotected with 30% sucrose and frozen. Sections 20 μm thick were cut and immunostained for c-Kit (a marker of ICC), neuronal (n) NOS, endothelial (e) NOS and PGP 9.5 (a marker of nerves). Double-immunostaining for c-Kit and NOS, and c-Kit immunohistochemistry combined with NADPHd histochemistry (a marker of NOS) were also performed. Numerous c-Kit positive cells in the muscle layers and myenteric regions of the stomach and intestine were found. Nerve fibers and neurons containing NADPHd diaphorase staining or nNOS immunoreactivity were also found in all the tissues. NADHPd labeling and eNOS immunoreactivity was found in blood vessels. Double labeling studies revealed the presence of NADPHd and nNOS labeled nerve fibers close to and surrounding some of the c-Kit positive cells. There was no co-localization of NADPHd, nNOS or eNOS with c-Kit labeled cells or their processes. Nor, was c-Kit immunoreactivity found in PGP 9.5 immunoreactive neurons and nerve fibers. These results suggest that ICC in the adult normal human gut do not contain neuronal or endothelial isoforms of NOS, unlike ICC in the canine colon. The sometimes close association between NOS containing nerve fibers and ICC in the human gut suggests that nitrergic nerves may modulate the activity of ICC or that ICC may play a role in modulating the NO signal produced by nitrergic nerves.

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● G3145

NITRIC OXIDE-MEDIATED SLOW IJPs IN THE CIRCULAR MUSCLE OF THE GUINEA-PIG ILEUM INVOLVE SUPPRESSION OF A CAMKII-DEPENDENT CHLORIDE CONDUCTANCE. Xue Dao He, Fivos Vogalis & Raj K. Goyal, Harvard Med. School & West Roxbury VAMC, 1400 VFW Pky, Boston MA 02132.

Electrical field stimulation (EFS) of intrinsic inhibitory motor nerves supplying the circular muscle layer of the guinea-pig ileum evokes a two-component inhibitory junction potential (IJP). The purinergic nerve-mediated initial or fast IJP is due to the opening of apamin-sensitive K^+ channels, while nitric oxide (NO) is responsible for the apamin-resistant slow IJP which may be generated by the cGMP-dependent suppression of a resting Cl^- conductance. In the present study we investigated the possibility that this Cl^- conductance is dependent on Ca^{2+} calmodulin-dependent protein kinase II (CaMKII) by testing the action of KN93, a potent inhibitor of CaMKII, on the nitrergic nerve-mediated slow IJP and the hyperpolarization elicited by diethylenetriamine (DNO), a stable NO donor. Full-thickness strips of guinea-pig ileum were pinned out in a recording chamber and perfused continuously with Krebs solution at 32°C. Intracellular recordings were obtained from cells in the circular muscle layer. Slow IJPs were evoked by EFS (4 x 0.5 ms pulses, 50 V) in the presence of apamin (0.3 μM) and substance P (1 μM). Slow IJPs were significantly decreased by pretreatment of tissues with 1-H[1,2,4] oxadiazolo [4,3-a] quinoxalin-1-one (ODQ, 6μM), a selective inhibitor of NO-stimulated guanylyl cyclase from 6.8 ± 0.3 mV to 0.3 ± 0.1 mV ($P<0.01$, n=15). In addition, ODQ decreased the DNO-induced hyperpolarization from 8.6 ± 0.5 mV to 0.6 ± 0.8 mV ($P < 0.01$, n=5). In another series of experiments, niflumic acid (NFA, 200 μM), a blocker of Ca^{2+}-activated Cl^- channels, also significantly decreased the amplitude of slow IJPs from 7.1 ± 0.5 to 0.3 ± 0.1 mV ($P<0.05$, n=5) and reduced the membrane hyperpolarization elicited by DNO (100 μM) from 8.5 ± 1.1 mV to 0.3 ± 0.2 ($P< 0.01$, n=5 cells). This suggests that NO inhibits a Ca^{2+}-activated Cl^- conductance through cGMP formation. To test the dependence of this conductance on CaMKII, tissue strips were pretreated with KN93 (20μM) for 20 min. This decreased the hyperpolarization elicited by DNO from 8.7 ± 0.4 mV to 0.4 ± 0.3 mV ($P<0.01$, n=8) and significantly reduced the slow IJP from 6.0 ± 0.4 mV to 0.2 ± 0.1mV ($P<0.01$, n=16). KN92 (20μM), an inactive structural analog of KN93 had no effect on either the DNO-mediated hyperpolarization or the slow IJP. In tissue strips untreated with apamin neither NFA nor ODQ nor KN93 had any effect on the purinergic fast IJP. The present results suggest that in the circular muscle layer of the guinea-pig ileum, the nitrergic nerve-mediated slow IJP and the DNO-induced hyperpolarization are generated by suppression of a Ca^{2+}-activated Cl^- conductance which may be constitutively activated by CaMKII.

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● G3146

INCREASED SMALL BOWEL BUT NOT COLONIC PERMEABILITY IN POST-INFECTIOUS IRRITABLE BOWEL SYNDROME. JM Hebden, PO Erah$^{\Delta}$, PE Blackshaw*, AC Perkins*, RC Spiller. Division of Gastroenterology, $^{\Delta}$Pharmaceutical Sciences, and *Medical Physics, University Hospital, Nottingham, NG7 2UH, UK.

Acute infectious diarrhoea is associated with abnormal mucosal permeability, which we speculated may persist in those who develop post-infectious irritable bowel syndrome (PIIBS).

Methods. Gut permeability was assessed by the lactulose-mannitol permeability test (small bowel) and by the urinary excretion of orally ingested Cr-51-EDTA (small and large bowel), in 17 patients with PIIBS and 12 healthy asymptomatic controls. The patients had suffered a preceding acute diarrhoeal illness (8 stool culture +ve), 9 to 47 months previously, and had negative haematological / biochemical / immunological screens together with radiological / endoscopic investigations. Urinary sugar excretion was measured by HPLC, and a L/M ration calculated. EDTA permeation was expressed as % of administered dose excreted in the urine.

Results. (mean ± SEM). The L/M ratio was elevated in patients (n=14) compared to controls (n=10); 0.10 ± 0.04 vs 0.03 ± 0.01 respectively, p=0.02. Those with a history < 12 months (n=4) tended to have greater ratios than those with longer histories (n=10); median (range) 0.19 (0.03-0.56) vs 0.04 (0.02-0.09) respectively, p=0.06. Although there was no difference in 24hr (whole gut) EDTA excretion (1.27 ± 0.13% vs 1.15 ± 0.10%, p=NS), the 0-6hr (predominantly small bowel) EDTA excretion was significantly greater in patients compared to controls (0.39 ± 0.03% vs 0.29 ± 0.03%, p<0.03).

Conclusions. A subgroup of patients with irritable bowel syndrome (post-infectious) demonstrate abnormal small bowel permeability, indicating an ongoing abnormality of mucosal function.

● G3147

ELECTROGASTROGRAPHY DIFFERENTIATES BETWEEN GASTROSTOMY TUBE FEEDING INTOLERANT VERSUS TOLERANT CHILDREN. J.B. Heikenen, S.L. Werlin, C.W. Brown, S.N. Reddy. Dept. of Pediatrics, Medical College of Wisconsin, Milwaukee, WI, Long Beach Memorial Hosp., Long Beach, CA.

BACKGROUND: Feeding disorders in children necessitating long term supplemental enteral nutrition are frequently managed by gastrostomy tube (GT) feedings. Some children develop gagging, retching, vomiting, or discomfort when fed by GT. Conventional medical management with changes in formula or their delivery, H2RA and prokinetic agents are not always successful. It is possible that intolerance of GT feedings reflects an underlying motility disorder of the foregut. **AIM:** To determine whether gastric electrical activity as measured by electrogastrography (EGG) is predictive of GT feeding intolerance in children. **METHODS:** Cutaneous EGG was performed on 20 GT feeding tolerant (FT) and 20 GT feeding intolerant (FI) children < 10yrs. Feeding intolerance was defined as gagging, retching, vomiting or postprandial discomfort requiring prokinetic medication and/or dietary manipulation. FT children tolerated bolus feedings in less than 30 min without symptoms or prokinetics. All patients were fasted at least 3 hrs and prokinetics withheld 48 hrs prior to EGG. Electrodes were applied over the epigastrium and a 30 minute baseline recording was performed. A GT feeding was administered and a 30 minute postprandial recording was obtained. The electrical signal was amplified, digitized, and stored for off line analysis. It was band pass filtered and visually edited for motion artifact. The dominant frequency (DF), rhythm index and power (P) were obtained for the fasting (NPO) and postprandial (PP) periods. Normogastria was defined as between 2.5-3.5 cpm, bradygastria < 2.5 cpm and tachygastria > 3.5 cpm. Differences between FT and FI groups were compared using the Student's t-test. **RESULTS**: In the FT group (12M) the mean age and duration of GT were 3.4 and 1.9 yrs respectively. Ten children had Nissens, 13 were neurologically impaired. In the FI group (9M) the mean age and duration of GT were 2.2 and 1.4 yrs. Nine children had Nissens, 18 were neurologically impaired. A greater percentage of the children in the FI group were neurologically impaired and significantly younger than those in the FT group (p<0.03).

	Dominant Freq. (cpm)		Bradygastria (%)		Normogastria (%)		Tachygastria (%)		Δ P (dB)
	NPO	PP	NPO	PP	NPO	PP	NPO	PP	
FT	3.5	3.6	13.2	10.0	42.5	47.8	46.8	41.6	+0.94
FI	3.7	3.7	12.6	13.9	45.0	43.2	42.4	42.9	-0.38

The difference in Δ power was significant ($p < 0.01$). There were no significant differences in the remaining parameters. **CONCLUSIONS:** 1) Children who are GT feeding intolerant have an abnormal postprandial power change. 2) In neurologically impaired children EGG rhythm indices are not predictive of GT feeding intolerance.