in the presence of guanethidine (10 μM) and N ω-nitro-L-arginine methylester (L-NAME, 100 μM). L-732,138 (NK1 receptor antagonist, 10 μM) or atropine (1 μM) were used to record contractions driven primarily by acetylcholine or tachykinins, respectively. In addition, contractions elicited by exogenous substance P (SP, 10 μM) or carbachol (10 μM), in the presence of tetrodotoxin (1 μM), were assessed. Electrically evoked contractile responses (5 Hz) in the presence L-NAME were also recorded and expressed as % of contractions in the absence of L-NAME. Each value represents the mean ± S.E.M obtained from 6 experiments. Results. Electrically evoked cholinergic contractions were decreased in rats sacrificed at day 28 and 56 after 6-OHDA injection (77.2±6.3 and 53.8±0.1, respectively), in comparison with controls (99±6.51). Conversely, carbachol-evoked contractions were enhanced in rats with PD both at day 28 and 56 (81.5±3.4 and 98.2±3.8, respectively), as compared to controls (52.2±5.6). Electrically evoked tachykininergic contractions were enhanced both at day 28 (85.6±5.2) and 56 (67.3±3.3), in comparison with controls (43.6±6.5). Likewise, contractions elicited by exogenous SP in colonic preparations from PD rats were also increased both at day 28 (244±11.9) and 56 (188.2±13.9), as compared to controls (79.6±6.3). In the presence of L-NAME, electrically evoked contractions in controls were enhanced (+42.3±11.9%), while they were decreased in rats at day 28 and 56 after 6-OHDA injection (+29.1±4.4% and +15±5.3%, respectively). Conclusion. Experimental PD, elicited by nigro-striatal dopaminergic degeneration, is associated with changes in neurotransmitter pathways driving colonic excitatory and inhibitory motor functions: an impairment of nitrergic and cholinergic transmission occurs in concomitance with an enhancement of tachykininergic control. Such a shift takes place along with an up-regulation of the contractile responses mediated by muscarinic receptors on smooth muscle, which might be compensatory in nature.

Su2046

Delayed Gastrointestinal Transit in *OB/OB* Mice Correlates With Reduced Intestinal TLR4 Signaling and Loss of Nitrergic Neurons

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Background: Obesity is associated with alterations in gut microbiota and gastrointestinal motility and a prevalence of constipation. We recently reported that lack of TLR4 activation can lead to delayed colonic motility. Thus, the goal of this study was to investigate our hypothesis that obesity-associated constipation might involve reduced levels of TLR4 promotion of motility. **Methods:** Studies were performed in male, 20 week old *ob/ob* mice and their WT littermates. Small intestinal transit time was determined by calculating the geometric center (GC) of passage of a fluorescent-labeled marker. The serum endotoxin level was detected by using Limulus Amebocyte Lysate assay. Intestinal TLR4 expression was quantitated by qRT-PCR. Nitrergic neurons were assessed by NADPH-diaphorase staining of the proximal colon from WT and *ob/ob* mice. In addition, both WT and *ob/ob* mice were treated with broad spectrum antibiotics for 4 weeks, and the whole gut and distal gut transit was monitored. **Results:** At 20 weeks of age the *ob/ob* mice had significantly greater weights than their age matched lean controls (weight in gms: WT 28.79 ± 0.78; *ob/ob*: 66.53 ± 0.85, n=8, P<0.001). Whole gut transit was slower in the *ob/ob* compared to their lean controls as evidenced by a lower geometric center of dye distribution (WT: 4.05 ± 0.24; *ob/ob*: 2.87 ± 0.44, n=4). In addition, distal gut transit was also slower in the *ob/ob* compared to WT mice (WT: 3.98 ± 0.26; *ob/ob*: 2.48 ± 0.68, n=4). In WT mice treated with antibiotics the whole and distal gut transit was delayed similar to the *ob/ob* mice. Antibiotic treatment in the *ob/ob* mice did not cause a further delay in the transit (WT with antibiotics: 2.51 ± 0.26; *ob/ob* with antibiotics: 2.81 ± 0.04, n=4, P >0.05). Serum endotoxin levels were lower in the *ob/ob* compared to WT mice (WT: 3.063 ± 0.42; *ob/ob*: 1.868 ± 0.86, n=3). TLR4 expression was lower in the colon of *ob/ob* compared to WT mice (20% reduction). In *ob/ob* there was reduction in the number of nitrergic neurons compared to WT mice (WT: 131.18, *ob/ob*: 106.78, n=4, P<0.001). **Conclusions:** Our results demonstrate delayed intestinal motility in *ob/ob* mice. This was associated with reduced TLR4 signaling and a decrease in nitrergic neurons in the proximal colon of *ob/ob* mice. Together our data suggest that reduced TLR4 signaling in *ob/ob* mice can contribute to the delayed intestinal motility. Further, manipulation of gut microbiota with antibiotics resulting in reduced TLR4 ligand is associated with delayed intestinal transit in the WT but not the *ob/ob* mice. Our results demonstrate that changes in gut microbiota in obesity can contribute to the delayed gastrointestinal transit seen in obese mice. These studies can help identify novel targets for the treatment of gastrointestinal motility disorders in obesity. Supported by NIH-RO1 (DK080684) and VA-MERIT award

Su2047

The Effect of 5-Hydroxytryptamine1a (5-HT1A) Receptor Agonist, Buspirone on Gastric Accommodation of Guinea Pig

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Gastric accommodation is active relaxation of gastric fundus according to food ingestion, and its impairment is an important pathophysiologic factor associated with functional dyspepsia. However, there are few therapeutic agents which could improve impaired gastric accommodation in clinical practice. Buspirone is 5-HT1a agonist which is commonly used as anxiolytic. There was a previous report that buspirone induced gastric relaxation in barostat study of healthy volunteers, and some animal experiments indicated that 5-HT1 receptor is involved in gastric accommodation. So, we aimed to investigate the effect of 5-HT1a receptor agonist, buspirone on gastric accommodation of guinea pig. Firstly, immunohistochemical stains for 5-HT1a receptor could confirm the expression of 5-HT1a receptor in guinea pig stomach. Secondly, muscle strips of fundic circular proper muscle(10mm×3mm), obtained from 300-350 weighed guinea pigs, were tested for electrical field stimulation(EFS) induced relaxation in tissue chamber filled with Krebs'-Henseleit solution. After High K+(25mM) induced pre-contraction under non-adrenergic & non-cholinergic condition(by atropine and guanethidine pretreatment), the EFS-induced relaxation of fundic muscle strips were significantly inhibited by WAY-100635(5-HT1a antagonist) by dose-dependent manner, and this inhibition was significantly ameliorated by pretreatment of buspirone. Thirdly, an in vivo gastric tone measurement experiment was done. After laparotomy under anesthesia, an indwelling polyethylene balloon was placed in gastric fundus of guinea pig. After recovery for 7 days, gastric tone was measured in conscious state, by distending the bag with air infusion and recording changes in intrabag pressure at various volumes. Pretreatment with buspirone significantly decreased intragastric pressure, compared with vehicle. But WAY-100635 pretreatment could block the buspirone-induced decrease of gastric pressure. In conclusion, this study could confirm the relaxation effect of 5-HT1a agonist buspirone on gastric fundus by both in vitro, and in vivo model. Such 5-HT1a agonist might be a possible therapeutic agent for functional dyspepsia with impaired gastric accommodation.

Su2048

Interstitial Cells of Cajal Integrate Excitatory and Inhibitory Neurotransmission With Intestinal Slow Wave Activity

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Introduction: The enteric nervous system contains excitatory and inhibitory neurons which control contraction and relaxation of smooth muscle cells and gastrointestinal motor activity. Interstitial Cells of Cajal (ICC) act as pacemaker in the gastrointestinal (GI) tract and orchestrate GI motility by generating slow waves of depolarisation which induce rhythmic contraction of smooth muscle cells. A role of ICC for excitatory and inhibitory neurotransmission is highly controversial and hampered by the lack of genetically defined models enabling time-specific targeting of ICC in adult animals. Methods: To analyse ICC function in adult mice in vivo, we generated a c-KitCreERT2 knock-in allele at the endogenous c-Kit locus. This mouse line enables for the first time genetic manipulation of ICC at defined time points during development and in adults in vivo. With the help of this novel model, we analyzed the specific role of ICC in enteric excitatory and inhibitory neurotransmission by genetic loss of function studies. We crossed c-KitCreERT2/+ mice with conditional LSL-R26DTA/+ animals, which carry a latent diphtheria toxin A (DTA) expression cassette to deplete ICC. To test whether ICC modulate inhibitory neurotransmission, we deleted cGMP-dependent protein kinase I (Prkg1), a central mediator of the non-adrenergic, non-cholinergic neurotransmission, in ICC by using floxed Prkg1lox/lox animals. Results: Tamoxifen induced depletion of more than 50% of the ICC network was observed in c-KitCreERT2; LSL-R26DTA/+ mice three days after treatment. ICC network disruption in healthy adult animals resulted in severely disturbed GI motility with significantly increased GI transit time. Importantly, all parts of the GI tract were equally affected by ICC depletion. The disturbed motility is caused by dysrhythmic spontaneous phasic myogenic contractions and lack of slow-wave type electrical activity in circular small intestinal smooth muscle cells. Organ bath experiments showed a significantly reduction of neuronal induced colonic smooth muscle contractions after electrical field stimulation in tamoxifen treated animals. A loss of Prkg1 expression in ~40% of all ICC resulted in disturbed GI motility and abolished specifically the NO-dependent component of the inhibitory junction potential in colonic circular smooth muscle cells. Conclusions: Time specific inducible depletion of the ICC network is feasible in adult animals using the novel c-KitCreERT2 expression model. In addition, we demonstrate for the first time a role for ICC in excitatory and inhibitory neurotransmission in phenotypic normal adult animals.

Su2049

Involvement of the P2X7 Purinergic Receptor in Colonic Motor Dysfunction Associated With Bowel Inflammation in Rats

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Introduction: Purinergic signalling plays a pivotal role in the physiological regulation of several enteric functions, as well as in the modulation of immune/inflammatory cell activity. Recent evidence has shown an active involvement of the purinergic P2X7 receptor (P2X7R) in the fine tuning of immune functions, as well as its critical role in driving enteric neuron apoptosis under intestinal inflammation. However, the participation of this receptor pathway in the regulation of enteric neuromuscular functions remains undetermined. Aims: This study investigated the role of P2X7Rs in the control of colonic motility, both under normal conditions and in the presence of experimental colitis. Methods: Colitis was induced by intrarectal administration of 2,4-dinitrobenzenesulfonic acid (DNBS) in adult male Sprague-Dawley rats. Six days after colitis induction, colonic longitudinal muscle strips (LMS), obtained from normal or inflamed rats, were suspended in organ baths, containing Krebs solution additioned with NK 1, 2 and 3 receptor antagonists, and connected to isometric transducers to record atropine-sensitive cholinergic motor activity. The effects of A804598 (selective P2X7R antagonist; 0.001-100 μM) and BzATP (selective P2X7R agonist; 0.001-10 μM) were tested on contractions evoked by electrical stimulation (ES: 0.5 ms, 28 V, 10 Hz), delivered as single train (sES) or repeated every 60 s (rES), or by carbachol (1 μM) in the presence of tetrodotoxin. Results: In normal LMS, A804598 induced a negligible enhancing effect on sES-induced contractions (+7.8±3.5% at 0.1 μM), while a significant increase in the contractile responses elicited by sES was recorded in LMS from inflamed animals (+42.5±3.9% at 0.1 μM). Incubation of LMS with the adrenergic blocker guanethidine (10 μM) did not affect the potentiating effect exerted by A804598 in the presence of colitis. In the presence of N ω-propyl-L-arginine (NPA, inhibitor of neuronal nitric oxide synthase), which per se increased the sES-induced contractions in both LMS preparations (+15.1±5.5% in normal and +143.5±9.5% in inflamed animals), the A804598 effects were lost. The pharmacological activation of P2X7Rs with BzATP did not significantly affect the contractions to rES in normal LMS (Emax= -10.2±1.9%), while a marked reduction was recorded in LMS from animals with colitis (Emax= -30.5±2.2%). The inhibitory effect of BzATP was antagonized by A804598, and it was also markedly blunted by NPA. Both P2X7R ligands did not affect carbachol-induced contractions. Conclusions: The purinergic system contributes to functional neuromuscular changes associated with bowel inflammation through the involvement of P2X7Rs, which modulate the activity of excitatory cholinergic nerves via a facilitatory control on inhibitory nitrergic pathways.