Divergent effects of exendin-4 and interleukin-6 on rat colonic secretory and contractile activity are associated with changes in regional vagal afferent signaling

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Abstract

Background: The pro-inflammatory cytokine, interleukin (IL)-6 is elevated in individuals with the functional bowel disorder, irritable bowel syndrome (IBS). IL-6 can independently modify intestinal secreto-motor function, thereby contributing to IBS pathophysiology. Additionally, hormonal changes may underlie symptom flares. Post-prandial exacerbation of IBS symptoms has been linked to secretion of the incretin hormone, glucagon-like peptide-1 (GLP-1), which can also influence colonic secreto-motor activity. This study aimed to ascertain if the effects of GLP-1 on colonic secretory and contractile activity was impacted by elevated IL-6 levels and if sensory signals regarding such changes were reflected in altered vagal afferent activity.

Methods: Colonic secretory currents and circular muscle contractile activity was investigated in Sprague Dawley rats using Ussing chamber and organ bath electrophysiology. Regional afferent signaling was assessed using extracellular electrophysiological recordings from colonic vagal afferents.

Key Results: Application of the GLP-1 receptor agonist, exendin-4 (Ex-4) in the presence of IL-6 potentiated colonic secretory currents and transepithelial resistance. Vagal afferent fibers originating in the submucosal layer exhibited larger responses to Ex-4 when IL-6 was also present. In contrast, co-application of Ex-4 and IL-6 to gut-bath chambers suppressed circular muscle contractile activity. The activity in extrinsic afferents originating in the colonic myenteric layer was similarly suppressed.

Conclusions & Inferences: Application of Ex-4 in the presence of IL-6 had divergent modulatory effects on colonic secretion and contractile activity. Similar patterns were observed in vagal afferent signaling originating in the submucosal and myenteric neuronal layers, indicating regional afferent activity reflected immune- and endocrine-mediated changes in colonic function.

KEYWORDS
enteric nervous system, glucagon-like peptide-1, interleukin-6, smooth muscle, vagus
1 | INTRODUCTION

Irritable bowel syndrome (IBS) is characterized by intestinal dysfunction. Abdominal pain and altered bowel habit are prevalent symptoms of this heterogeneous bowel disorder. We are beginning to understand the pathophysiological changes underlying IBS, with evidence of immune activation and altered neuroendocrine signaling detected in patients. Circulating levels of pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, and TNF-α are altered. We have elucidated the neuromodulatory actions of IL-6 in enteric neurons, which underpins changes in intestinal secretion and smooth muscle contractile activity. However, changes in hormonal secretion also contribute to the etiology of IBS. Indeed, in the context of elevated circulating levels of pro-inflammatory cytokines, we have observed additive effects of the stress hormone, corticotrophin-releasing factor on colonic function.

A commonly reported symptom of IBS is the exacerbation of symptoms such as bloating, abdominal pain, and diarrhea following a meal. This coincides with nutrient-induced basolateral secretion of glucagon-like peptide-1 (GLP-1) from polarized enteroendocrine L-cells that are embedded throughout the intestinal epithelium. This incretin hormone stimulates the pancreatic release of insulin but also has paracrine effects locally in the gut. GLP-1 suppresses gastric emptying, small intestinal secretion, and the migrating motor complex. In contrast to the inhibitory effects in the proximal intestine, GLP-1 has been found to enhance colonic transit via a vagally mediated signaling mechanism. Given the acute exacerbation of IBS symptoms in the post-prandial period, and GLP-1-evoked changes in motility observed in patients with IBS as well as healthy individuals, this gut hormone may be important in mechanisms underlying bowel dysfunction. Indeed, further evidence to support this supposition includes suppressed circulating levels of GLP-1 in diarrhea-predominant IBS patients and the improvement in visceral pain and gut spasms in IBS patients treated with a GLP-1 mimic. We have reproduced the positive effects of GLP-1 on gut function in the Wistar-Kyoto rat model of IBS and used healthy Sprague-Dawley rats colons to further explore the mechanisms of action underlying these beneficial effects. We found that exendin 4 (Ex-4), a longer-lasting GLP-1 receptor agonist, decreased the amplitude of circular smooth muscle contractions, increased the amplitude of colonic secretory currents, and decreased barrier permeability.

In the context of elevated circulating concentrations of IL-6 in IBS patients and animal models of IBS, and given that IL-6 can induce GLP-1 secretion, particularly in conditions associated with immune activation, we hypothesized that changes in colonic secreto-motor function will be further modified by enteric secretion of GLP-1. Indeed, we have observed that addition of Ex-4 in the presence of IL-6 had divergent effects on the enteric plexi. Calcium responses evoked by Ex-4 in the presence of IL-6 in submucosal neurons were enhanced, whereas calcium responses in myenteric neurons were suppressed in comparison with Ex-4 alone. The aims of this study were to determine whether the modulatory effects of Ex-4 on colonic secretory and contractile function were impacted by the presence of IL-6. Moreover, we sought to investigate whether vagal afferents acted as signaling conduits for relaying such information to the central nervous system (CNS).

2 | METHODS

2.1 | Ethical approval

All experiments were in full accordance with the European Community Council Directive (86/609/EEC) and local guidelines from University College Cork animal ethical committee (#2011/015).

2.2 | Animals and tissue collecting

Male Sprague-Dawley rats (8–12 weeks old) purchased from Envigo, Derbyshire, UK, were group-housed 5 per cage and maintained on a 12/12 h dark-light cycle (08.00–20.00) with a room temperature of 22 ± 1°C. Given that hormonal cycles in the female are associated with exacerbation of symptoms, we used male Sprague-Dawley rats in this study to avoid the additional complexity of cycling female hormone levels when trying to understand the contribution of GLP-1 to bowel dysfunction. Animals were permitted at least a week to acclimatize to their new environment prior to experimentation. Food and water were available ad libitum. Rats were euthanized by CO2 overdose and perforation of the diaphragm. A section of transverse colon, cut 5 cm proximal from the anus, was excised and stored in cold Krebs-buffered saline containing mmol L−1: 117 NaCl, 4.8 KCl, 2.5 CaCl2, 1.2 MgCl2, 25 NaHCO3, 1.2 NaH2PO4, and 11 d-glucose (pH 7.4). The mucosa was
removed to expose the submucosal plexus. To expose myenteric neurons, the circular muscle layers were peeled away using forceps leaving a longitudinal muscle myenteric plexus preparation. Sections of colon stripped of the mucosa and orientated to measure circular muscle contractile activity were used for gut bath studies, whereas mucosal-submucosal tissue preparations were used for experiments in the Ussing chambers. The dissection technique used for the colon-vagal nerve tissue preparation is described in detail below.

2.3 | Ussing chamber electrophysiology

Mucosa-submucosal preparations of transverse colon were mounted in Ussing chambers (exposed area of 0.12 cm²) with 5 ml of Krebs saline solution (95% O₂/5% CO₂, 37°C) in the basolateral and luminal reservoirs. Tissues were voltage-clamped at 0 mV using an automatic voltage clamp (EVC 4000, World Precision Instruments), and the short-circuit current (Isc) required to maintain the 0 mV potential was monitored as a recording of the net active ion transport across the epithelium. Experiments were carried out simultaneously in all chambers and connected to a PC equipped with DataTrax II software (World Precision Instruments). This software was used to measure the peak response, and resistance was calculated using Ohms law. Following mounting, tissue was allowed to equilibrate (~1 h). Reagents (exendin-4 (Ex-4, 10µM, Tocris: cat. No. 1933), capsaicin (1 µM, Sigma-Aldrich, cat. No.: M2028), carbachol (1 µM, Sigma-Aldrich, cat. No.: Y0000113), interleukin-6 (IL-6, 1 nM, Tocris: cat. No.: 1078), and veratridine (10 µM, Sigma-Aldrich, cat. No.: V5754) were added to the basolateral chamber.

2.4 | Colonic-Afferent nerve electrophysiological recordings

The detailed description of the ex vivo dissection technique and recording of colonic-afferent nerves has been previously reported. In brief, a segment of the esophagus with attached posterior vagus nerve was excised from an adult Sprague-Dawley rat. Maintaining an intact neural connection to the esophagus, a segment of transverse colon (5 cm from the anus) with attached inferior and superior mesenteric ganglia, celiac ganglia, and vagus nerve were placed in a recording chamber. Adjacent Sylgard-lined chambers allowed the afferent nerves to be isolated from the colon. The colon was opened, mucosal side up and the vagus nerve was carefully threaded through to the adjacent chamber and the gap was sealed with petroleum jelly. Both chambers were superfused with 5% CO₂/95% O₂ bubbled Krebs-buffered saline maintained at 37°C.

For studies examining afferents originating in the submucosal or myenteric plexi, the colon was opened longitudinally, off center to the mesentery border to orientate the intact nerve fibers to the edge of the opened tissue. The mucosa was carefully peeled away using forceps to expose the submucosal neurons. To expose myenteric neurons, the submucosal layer and circular muscle fibers were removed. Reagents were added to the colonic bath in random order to prevent potential confounding results due to desensitization.

Multi-unit neural activity was recorded using platinum bipolar recording electrodes (World Precision Instruments) attached to a Power lab device (AD Instruments). Reagents were directly applied to the colonic bath. Nerve activity was viewed and analyzed with Chart 7 (AD Instruments). Changes in multi-unit neural activity in the vagal nerve are presented as frequency from raw traces. Both raw and rectified traces are presented.

2.5 | Gut bath electrophysiology

Sections of colon were opened along the mesenteric border, and the mucosa was removed. Tissue was suspended transversely to measure circular muscle contractility. Colonic sections were suspended from a tension transducer under 1 g of tension in a water-jacketed tissue bath, maintained at 37°C in Krebs-buffered saline, and allowed to equilibrate for up to an hour. Colonic sections were stimulated with the cholinergic agonist, carbachol (1 µM, Sigma-Aldrich, 5 min) to stimulate a maximal contractile response. Carbachol was added at the start and end of experiments to ensure tissue viability. Ex-4 and IL-6 were added to the bath in a random order to ensure no decline in muscle responsiveness occurred over time. Baseline contractile activity was recorded prior to addition of reagents, and for analysis, changes in activity were compared to the new baseline. Before addition of the next reagent, the bath was flushed three times with Krebs saline solution and the tissue was allowed re-stabilize by recovering typical baseline contractions. No further reagents were added until the baseline was restored. Contractile changes in isolated muscle strips were recorded via a mechanical transducer and Powerlab system and LabChart7 (all AD Instruments Inc.). The rectified trace was calculated by computing the integral of the raw data.

2.6 | Statistical analyses

Data were analyzed using GraphPad Prism for windows (version 6, GraphPad Software). The data are represented as data plots with mean ± the standard deviation. One-way ANOVA with Tukey multiple comparison post hoc tests or paired t tests, as appropriate were used to compare data. p ≤ 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Colonic secretion was enhanced upon exposure to ex-4 in the presence of il-6

As application of Ex-4 in the presence of IL-6 resulted in enhanced calcium responses in submucosal neurons, Ussing chamber studies were used to investigate the effects of Ex-4, either alone or with IL-6.
on short-circuit current ($I_{sc}$), a measure of net ion transport across the colonic epithelium. Separately, addition of IL-6 ($103.6 \pm 39.2$, $n = 7$) and Ex-4 $^{30}$ ($65.04 \pm 26.3$, $n = 7$) to the basolateral reservoir resulted in secretory currents with amplitudes larger than saline controls ($16.87 \pm 12.7 \mu A/cm^2$, $n = 7$). The secretory currents evoked by Ex-4 with IL-6 ($161 \pm 108.5 \mu A/cm^2$, $n = 7$) were larger than currents evoked by Ex-4 alone ($p = 0.0016$, Figure 1A). However, the current induced by IL-6 (1 nM) was larger than that evoked by Ex-4 (10 µM, $p = 0.0047$).

The neurally regulated secretory current evoked by application of the Na$^+$ channel agonist, veratridine (10 µM, $10.49 \pm 6.9 \mu A/cm^2$, $n = 6$), which causes sustained neuronal depolarization, was potentiated by both Ex-4 ($129.9 \pm 95.2 \mu A/cm^2$, $n = 6$) and IL-6 ($289.1 \pm 64.4 \mu A/cm^2$, $n = 6$), although the response generated by IL-6 was larger ($n = 6$, $p = 0.0003$). Addition of Ex-4 in the presence of IL-6 was not different to Ex-4 alone ($233.1 \pm 152.7 \mu A/cm^2$, $n = 6$, $p > 0.05$, Figure 1B).

The pro-secretory current evoked by the cholinergic agonist, carbachol (1 µM, $12.1 \pm 10.0 \mu A/cm^2$, $n = 6$), was not significantly modified by addition of either Ex-4 ($94.3 \pm 102 \mu A/cm^2$, $n = 6$) or IL-6 ($136.3 \pm 97.3 \mu A/cm^2$, $n = 6$) alone. However, the current was significantly potentiated when both Ex-4 and IL-6 were added together ($325.7 \pm 281.2 \mu A/cm^2$, $n = 6$, $p = 0.0174$). The amplitude of the secretory current evoked by carbachol in the presence of Ex-4 and IL-6 was larger than the response evoked by Ex-4 alone ($p < 0.05$, Figure 1C).

Capsaicin (1 µM), an afferent nerve stimulant, evokes a biphasic secretory and anti-secretory response in colonic tissue. In the initial secretory phase, when both Ex-4 and IL-6 were added together, the capsaicin pro-secretory current was enhanced ($194.2 \pm 143.6 \mu A/cm^2$, $n = 7$) as compared to the saline control ($7.7 \pm 5.91 \mu A/cm^2$, $n = 7$, $p = 0.015$). However, this was not different to the enhanced currents evoked by IL-6 ($150.3 \pm 108.7 \mu A/cm^2$, $n = 7$) or Ex-4 ($149.8 \pm 107.7 \mu A/cm^2$, $n = 7$) alone ($n = 7$, Figure 1D). The anti-secretory phase of the current evoked by capsaicin ($-19.85 \pm 19.03 \mu A/cm^2$, $n = 6$) was not significantly changed by co-application of Ex-4 and IL-6 ($-314.1 \pm 309 \mu A/cm^2$, $n = 6$) nor were any differences noted between Ex-4 ($-130.2 \pm 106.3 \mu A/cm^2$, $n = 6$) or IL-6 ($-231.3 \pm 205.5 \mu A/cm^2$, $n = 6$, $p = 0.0935$, $n = 6$, Figure 1D).

As previously reported,$^{30}$ over the course of the experiment (60–90 min), the relative change in transepithelial resistance (TER), a measure of gut leakage, increased in tissues continuously exposed to Ex-4 ($389 \pm 108.8 \mu A/cm^2$) compared to the pre-treated measurement ($374.9 \pm 5.8 \mu A/cm^2$, $n = 8$, $p = 0.002$). Tissue incubated with saline alone showed no change over the course of the experiment ($375.2 \pm 3.15$ vs $375.5 \pm 5.83 \mu A/cm^2$, $n = 8$, $p = 0.89$).

**Figure 1** - Co-application of Ex-4 and IL-6 potentiated colonic secretory currents. (A) The representative secretory traces from Ussing chambers and the associated data plots illustrate the secretory currents ($I_{sc}$) evoked by the GLP-1R agonist, exendin 4 (Ex-4, 10 µM), interleukin-6 (IL-6, 1 nM), or Ex-4 in the presence of IL-6 ($n = 7$). (B) The traces and data plots illustrate the modulatory effects of Ex-4, IL-6 and application of both together on currents evoked by the sodium channel agonist, veratridine (10 µM, $n = 8$); (C) the cholinergic agonist, carbachol (1 µM, $n = 8$) and (D) the sensory nerve stimulant, capsaicin (1 µM, $n = 8$). (E) The data plot shows transepithelial resistance (TER) before and after exposure to Ex-4, IL-6, or both reagents. *$p < 0.05$, **$p < 0.01$.

### 3.2 Activity in vagal afferents originating in the submucosal plexus is enhanced by ex-4 and il-6

An ex vivo preparation of rat colon with intact vagal afferents was used to determine the effects of Ex-4 and IL-6 on regional vagal afferent activity. In this colonic preparation, the submucosal neurons and the extrinsic afferent nerve endings innervating this gut layer were exposed to the receptor agonists. Consistent with previous reports,$^{31}$ in the absence of stimulation, baseline afferent activity was low. Both Ex-4 (10 µM, 3 min, $4.19 \pm 0.77$ peaks/min) and IL-6 (1 nM, 3 min, $1.9 \pm 1.67$ peaks/min) stimulated robust but short-lived increases in vagal afferent activity ($n = 5$). Neuronal activity evoked by Ex-4 was greater than that evoked by IL-6 ($p < 0.05$). However, application of Ex-4 in the presence of IL-6 ($5.79 \pm 1.1$ peaks/min) was not significantly different to that evoked by Ex-4 alone ($p = 0.015$, Figure 2).

### 3.3 Application of ex-4 in the presence of il-6 had a suppressive effect on colonic contractile activity

We previously found that application Ex-4 in the presence of IL-6 suppressed the calcium response in myenteric neurons, when compared to responses evoked by either Ex-4 or IL-6 alone.$^{7}$ To assess whether this observation at the cellular level had functional consequences, the receptor agonists were applied to a colonic smooth muscle tissue preparation laterally orientated to assess circular muscle contractile activity. Baseline colonic circular smooth muscle contractions were regular in the Sprague-Dawley rat tissue, with reasonably consistent amplitude and frequency of contractions (Figure 3). The cholinergic agonist, carbachol (1 µM), was applied to the tissue to evoke a maximal response and ensure tissue viability. All tissues included in analysis responded to carbachol (0.062 ± 0.06, $n = 4$). Ex-4 (10 µM, 20 min) induced a robust contractile response as compared to baseline (0.0085 ± 0.04, $n = 4$) with increased frequency of contractions and a slowly developing increase in tone. IL-6 (1 nM, 20 min) modified contractile activity (0.0053 ± 0.001, $n = 4$) resulting in lower amplitude contractions as compared to baseline. Contractile activity was further suppressed when Ex-4 was added to the gut bath with...
IL-6 (0.004 ± 0.002, n = 4, p = 0.0345, Figure 3). When contractile activity evoked by Ex-4, IL-6, or Ex-4 in the presence of IL-6 was normalized to the maximal contraction evoked by carbachol, we similarly observed suppression of the Ex-4-evoked response when IL-6 was co-applied (p = 0.0402, data not shown).

### 3.4 Activity in vagal afferents originating in the myenteric plexus is suppressed by application of ex-4 in the presence of il-6

Baseline vagal nerve activity was quiescent in the absence of stimulation of nerve fibers originating in the myenteric plexus layer. However, a robust increase in vagal afferent activity was observed when Ex-4 (10 µM, 3 min, 3.79 ± 0.53 peaks/min) was applied to the colonic myenteric layer. The short-lived response decreased rapidly upon washout. IL-6 (1 nM, 3 min) also had neurostimulatory effects (1.79 ± 0.67 peaks/min) although the response was less than that observed for Ex-4 (n = 4 colonic vagal afferent preparations, p = 0.004). Following a similar pattern to the effects of Ex-4 and IL-6 on circular muscle activity, when Ex-4 was applied to the colonic myenteric plexus in the presence of IL-6, the response was decreased (1.49 ± 0.6 peaks/min), as compared to Ex-4 alone (p = 0.003, Figure 4).

### 4 DISCUSSION

Circulating factors can be more than simple biomarkers of a particular disorder, and in the case of pro-inflammatory cytokines and intestinal hormones, we have previously provided evidence of their neuromodulatory actions in enteric neurons. Moreover, we have reported crosstalk between endocrine and immune factors in submucosal and myenteric neurons, which resulted in functional changes. Such observations are particularly relevant to our understanding of the pathophysiology of bowel disorders, such as IBS, where cytokine and endocrine profiles differ to those of healthy individuals. Moreover, in an animal model of bowel dysfunction, we have observed different sensitivities to gut-modulating factors. IL-6-mediated potentiation of colonic secretion in healthy Sprague-Dawley rats was absent in stress-sensitive Wistar-Kyoto rats, which manifest IBS-like symptoms. Furthermore, in vivo investigations in Wistar-Kyoto rats found that Ex-4 alone normalized stress-induced defecation rates, but there were no additional effects of IL-6. The current study has examined potential cross-communication between agonists for the IL-6 and GLP-1 receptors, both of which are expressed on intrinsic and extrinsic gut neurons. We also assessed how such interactions could modify colonic secretory and contractile function and we investigated if regional afferent signaling from the colon reflected the evoked changes in colonic function.

The findings from this study build upon our recent report of divergent effects of IL-6 and the GLP-1R agonist, Ex-4, in submucosal and myenteric neurons. Consistent with those findings in the submucosal plexus, the neuronal regulators of absorptive and secretory activity in the gut, where calcium responses evoked by Ex-4 were potentiated when IL-6 was co-applied, we observed that application of Ex-4 in the presence of IL-6 stimulated a secretory current from the colonic epithelium that was larger than
that evoked by Ex-4 alone. This experimental design mimics post-prandial GLP-1 secretion in an individual with IBS who has elevated circulating IL-6. Aggravation of symptoms following a meal is commonly reported in IBS patients.\textsuperscript{20–22} To investigate further, the role of neuronal control in colonic secretion, veratridine, a lipid-soluble steroidal alkaloid which results in neuronal depolarization was applied to evoke a secretory current. Both Ex-4\textsuperscript{30} and IL-6\textsuperscript{35} independently enhanced veratridine-evoked secretory currents; however, despite the modulatory effect of IL-6 being larger than that of Ex-4, no additional increase was observed when both reagents were applied together. This could be due to different sub-populations of submucosal neurons being activated by either IL-6 or Ex-4. Indeed, we have reported that receptors for IL-6 and GLP-1 are co-expressed only in ~30% of neurons.\textsuperscript{29} Carbachol evoked a secretory current by activating pro-secretory cholinergic submucosal neurons\textsuperscript{36} and this was enhanced when Ex-4 was applied in the presence of IL-6. This response that was greater than that evoked by Ex-4 alone, suggesting that cholinergic neurons may be key to the enhanced secretory response evoked by co-application of IL-6 and Ex-4.

Capsaicin, which activates afferent nerves expressing transient receptor potential type V1 (TRPV1) ion channels,\textsuperscript{37} evoked a characteristic secretory and anti-secretory current.\textsuperscript{38} More than two thirds of vagal afferent fibers express TRPV1\textsuperscript{39} and are critical to relaying interoceptive signals to the CNS. Local increases in pro-inflammatory cytokines activate capsaicin-sensitive vagal afferents, which, in turn activated neurons in the nucleus tractus solitarius.\textsuperscript{40} Vago-vagal signaling can subsequently stimulate descending cholinergic anti-inflammatory reflexes, reducing inflammation and disease activity in various animal models of intestinal inflammation.\textsuperscript{41} This protective physiological response could be an important homeostatic response to symptom flares in IBS. In our studies, IL-6 and Ex-4 individually modified the capsaicin-evoked current, but no summative effect was noted in either the secretory or anti-secretory phases when Ex-4 was co-applied with IL-6. In studies examining transepithelial resistance over the course of the experiments (~90 min), IL-6 decreased epithelial leakiness indicating a potential protective response to inflammatory mediators at a local level. Ex-4 also increased TER over the course of the experiment; however, no further increase in TER was observed.
when both Ex-4 and IL-6 were added to the basolateral chamber, suggesting that IL-6 was the key driver of this response.

GLP-1 can bind to peripheral vagal receptors and receptors in the central nervous system and Bohorquez et al. demonstrated a physical connection between GLP-1 secreting L-cells, which are embedded in the epithelium and afferent fibers. This indicates the existence of a neural pathway to inform the CNS of changes in gut function. Others have suggested that intrinsic primary afferent neurons (IPANs) may act as the starting point in gut-brain signaling, although no immunoreactivity for putative IPANs was detected in the submucosal plexus of mouse small intestine. Submucosal neurons and vagal fibres express GLP-1 receptors. By applying Ex-4 to exposed submucosal neurons and extrinsic afferent endings terminating in this gut layer, we mimicked paracrine activation of gut receptors by basolaterally released GLP-1. Ex-4 stimulated an increase in the frequency of action potentials recorded from vagal afferents, which could be through direct activation of vagal afferents or indirectly through activation of submucosal neurons.

Communication between the immune system and the CNS is critical to initiating the inflammatory reflex, a physiological response where vagal afferents are stimulated by pro-inflammatory cytokines resulting in activation of vagal afferents which regulate the release of pro-inflammatory mediators. Vagal afferents express receptors for pro-inflammatory cytokines such as TNFα and IL-1β, and in our studies, we found that IL-6 also stimulated vagal afferent activity, although the response was less than that evoked by Ex-4. When Ex-4 was applied in the presence of IL-6, there was an increase in frequency but it was not significantly greater than Ex-4 alone, which is similar to what we observed with veratridine-evoked neurally regulated secretory currents. However, a caveat to these compound extracellular recordings is that signals are incorporated from multiple afferent fibers. Thus, it is not possible to determine whether the same fibers are being stimulated by both Ex-4 and IL-6. Moreover, further studies are required to determine where activated fibers terminate and if there is further interaction at the level of the brainstem.

Coordinated interactions between myenteric neurons, interstitial cells of Cajal and smooth muscle cells underlie intestinal motility, and regulation is made more complex by the modulatory effects of various hormones and inflammatory mediators. Smooth muscle hypercontractility and heightened central perception of visceral pain underlie abdominal pain symptoms in IBS. Gut spasm is a debilitating symptom of IBS caused by contraction of circular smooth muscle which is regulated by myenteric neurons. Contrasting with the broadly summative effects on colonic secretion when Ex-4 was applied in the presence of IL-6, we found that contractile activity in colonic circular muscle was decreased when both agonists were present in comparison with the effects of Ex-4 alone. Contractile activity evoked by Ex-4 in colonic circular muscle resulted in an increase in the frequency of contractions and a slowly developing increase in smooth muscle tone, a finding that is consistent with previous reports of enhanced transit in the colon via a vagally mediated signaling mechanism evoked by GLP-1. IL-6 is a pleiotropic cytokine with variable effects on colonic contractility that appears to be dependent on the animal model. In Sprague-Dawley colonic tissue, IL-6 diminished smooth muscle activity as compared to baseline and suppressed the stimulatory effects of Ex-4, a finding that fits with cellular responses where co-application of IL-6 and Ex-4 suppressed calcium responses in myenteric neurons. Finally, to determine whether functional changes in contractile activity...
evoked by Ex-4 and IL-6 impacted on regional afferent activity, we examined vagal excitability in response to stimulation of the colonic myenteric plexus and associated afferent endings. Exposure of the myenteric layer to Ex-4 alone resulted in a robust increase in vagal firing. The response to IL-6 was significantly less, and furthermore, the presence of IL-6 suppressed Ex-4-evoked stimulation of vagal afferents originating in the myenteric neuronal plexus, thus following the same pattern as was noted in the modulation of colonic circular muscle contractile activity.

This latest study supports a growing body of data that has revealed complicated interactions between cytokines and hormones in the gut. Moreover, several physiological systems are involved in the diverse effects of endocrine and immune factors on gut function, which is likely to be a key factor underlying the heterogeneity of symptoms relating to the pathophysiology of functional bowel disorders. Circulating levels of IL-6 and GLP-1 differ between healthy individuals and IBS patients implicating these immune and endocrine factors in disruption of bowel function. Endogenous GLP-1 is thought to protect against harmful stimuli; indeed, a pro-inflammatory stimulus dependent upon IL-6 resulted in increased secretion of GLP-1 in both mice and humans. In IBS, decreased mucosal expression of GLP-1 receptors was correlated with abdominal pain severity and we have previously demonstrated that intraperitoneal administration of Ex-4 improved visceral pain sensitivity in an animal model of IBS.

From the myriad of neuropeptidergic factors in the gut, even when limited to just GLP-1 and IL-6 receptor agonists, we observed divergent functional outcomes in the submucosal and myenteric plexi. When both Ex-4 and IL-6 were present, the amplitude of colonic secretory currents and transepithelial resistance were potentiated. Vagal afferent activity originating in the submucosal layer also exhibited an enhanced response to Ex-4 and IL-6. In colonic circular muscle, application of Ex-4 in the presence of IL-6 suppressed contractile activity and vagal nerve activity was similarly suppressed when Ex-4 and IL-6 were co-applied to the colonic myenteric layer. This study provides further evidence that endocrine and immune factors can modulate gut function and supports the concept of interoceptive signaling via colonic vagal afferents.

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CONFLICT OF INTERESTS
The authors have no competing interests.

DECLARATION
RO’B and MMB performed the research and analyzed the data. DO’M designed the research study, wrote the paper, and secured funding.

DATA AVAILABILITY STATEMENT
All data sets are available upon request to the corresponding author.

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REFERENCES
1. Enck P, Aziz Q, Barbara G, et al. Irritable bowel syndrome. Nat Rev Dis Primers. 2016;2:16014.
2. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10(7):712-721.
3. Dinan TG, Quigley EM, Ahmed SM, et al. Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? Gastroenterology. 2006;130(2):304-311.
4. El-Salhy M, Seim I, Chopin L, Gundersen D, Hatlebakk JG, Hausken T. Irritable bowel syndrome: the role of gut neuroendocrine peptides. Front Biosci (Elite Ed). 2012;4:2783-2800.
5. Liebergrest T, Adam B, Bredack C, et al. Immune activation in patients with irritable bowel syndrome. Gastroenterology. 2007;132(3):913-920.
6. Aigbologa J, Connolly M, Buckley JM, O’Malley D. Mucosal tuft cell density is increased in diarrhoea-predominant irritable bowel syndrome colonic biopsies. Front Psychiatry. 2020;11:436.
7. O’Malley D, Liston M, Hyndl NP, Dinan TG, Cryan JF. Colonic soluble mediators from the maternal separation model of irritable bowel syndrome activate submucosal neurons via an interleukin-6-dependent mechanism. Am J Physiol Gastrointest Liver Physiol. 2011;300(2):G241-G252.
8. Buckley MM, O’Halloran KD, Rae MG, Dinan TG, O’Malley D. Modulation of enteric neurons by interleukin-6 and corticotropin-releasing factor contributes to visceral hypersensitivity and altered colonic motility in a rat model of irritable bowel syndrome. J Physiol. 2014;592(23):5235–5250.
9. O’Brien R, O’Malley D. The Glucagon-like peptide-1 receptor agonist, exendin-4, ameliorated gastrointestinal dysfunction in the Wistar Kyoto rat model of Irritable Bowel Syndrome. Neurogastroenterol Motil. 2020;32(2):e13738.
10. O’Malley D, Buckley MM, McKernan DP, Quigley EM, Cryan JF, Dinan TG. Soluble mediators in plasma from irritable bowel syndrome patients excite rat submucosal neurons. Brain Behav Immun. 2015;44:57-67.
11. O’Malley D, Cryan JF, Dinan TG. Crossstalk between interleukin-6 and corticotropin-releasing factor modulate submucosal plexus activity and colonic secretion. Brain Behav Immun. 2013;30:115-124.
12. O’Malley D, Dinan TG, Cryan JF. Altered expression and secretion of colonic interleukin-6 in a stress-sensitive animal model of brain-gut axis dysfunction. J Neuroimmuno. 2011;235(1-2):48-55.
13. O’Malley D, Dinan TG, Cryan JF. Interleukin-6 modulates colonic transepithelial ion transport in the stress-sensitive wistar kyoto rat. Front Pharmacol. 2012;3:190.
14. O’Malley D, Quigley EM, Dinan TG, Cryan JF. Do interactions between stress and immune responses lead to symptom exacerbations in irritable bowel syndrome? Brain Behav Immun. 2011;25(7):1333-1341.
15. Moloney RD, Johnson AC, O’Mahony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the microbiota-gut-brain axis in visceral pain: relevance to irritable bowel Syndrome. CNS Neurosci Ther. 2016;22(2):102-117.
16. Simren M, Mannson A, Langkilde AM, et al. Food-related gastrointestinal symptoms in the irritable bowel syndrome. Digestion. 2001;63(2):108-115.
17. Elliott RM, Morgan LM, Treder JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1 (7–36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. J Endocrinol. 1993;138(1):159-166.

18. Baldassano S, Wang GD, Mule F, Wood JD. Glucagon-like peptide-1 modulates neurally-evoked mucosal chloride secretion in guinea pig small intestine in vitro. Am J Physiol Gastrointest Liver Physiol. 2011;302(3):G352-G358.

19. Nakade Y, Tsukamoto K, Iwa M, Pappas TN, Takahashi T. Glucagon-like peptide-1 accelerates colonic transit via central CRF and peripheral vagal pathways in conscious rats. Auton Neurosci. 2007;131(1-2):50-56.

20. Cabre E. Irritable bowel syndrome: can nutrient manipulation help? Curr Opin Clin Nutr Metab Care. 2010;13(5):581-587.

21. Morcos A, Dinan T, Quigley EM. Irritable bowel syndrome: role of food in pathogenesis and management. J Dig Dis. 2009;10(4):237-246.

22. Ragnarsson G, Bodemar G. Pain is temporally related to eating but not to defaecation in the irritable bowel syndrome (IBS). Patients’ description of diarrhoea, constipation and symptom variation during a prospective 6-week study. Eur J Gastro Hepatol. 1998;10(5):415-421.

23. Hellstrom PM, Naslund E, Edholm T, et al. GLP-1 suppresses gastrointestinal motility and inhibits the migrating motor complex in healthy subjects and patients with irritable bowel syndrome. Neurogastroenterol Motil. 2008;20(6):649-659.

24. Schirra J, Houck P, Wank U, Arnold R, Göke B, Katschincki M. Effects of glucagon-like peptide-1(7-36)amide on antro-pyloro-duodenal motility in the interdigestive state and with duodenal lipid perfusion in humans. Gut. 2000;46(5):622-631.

25. Schirra J, Nicolaus M, Roggel R, et al. Endogenous glucagon-like peptide 1 controls endocrine pancreatic secretion and antro-pyloro-duodenal motility in humans. Gut. 2006;55(2):243-251.

26. Buckley MM, O’Brien R, Buckley JM, O’Malley D. GH5R-1 agonist sensitizes rat colonic intrinsic and extrinsic neurons to exenatide: a role in the manifestation of postprandial gastrointestinal symptoms in irritable bowel syndrome? Neurogastroenterol Motil. 2019:e13684.

27. Hellstrom PM, Hein J, Bytzer P, Bjornsson E, et al. Leptin modifies the postprandial and 24-h secretion patterns of pathogenic bacterial colonization in the gut. J Neuroimmunol. 2013;257(1-2):36-45.

28. Mosinska P, Salaga M, Fichna J. Novel investigational drugs for capsaicin-sensitive vagal afferent neurons contribute to the detection of pathogenic bacterial colonization in the gut. J Neuroimmunol. 2000;96(2):407-416.

29. Yarrow S, Ferrar JA, Cox HM. The effects of capsaicin upon electrogenic ion transport in rat descending colon. Naunyn Schmiedebergs Arch Pharmacol. 1991;344(5):557-563.

30. Li BY, Schild JH. Electrophysiological and pharmacological validation of vagal afferent fiber type of neurons enzymatically isolated from rat nodose ganglia. J Neurosci Methods. 2007;164(1):75-85.

31. Riley TP, Neal-McKinney JM, Buelow DR, Konkel ME, Simasko SM. Capsaicin-sensitive vagal afferent neurons contribute to the detection of pathogenic bacterial colonization in the gut. J Neuroimmunol. 2013;257(1-2):36-45.

32. Kedees MH, Guz Y, Grigoryan M, Teitelman G. Functional activity of murine intestinal mucosal cells is regulated by the glucagon-like peptide-1 receptor. Peptides. 2013;48:36-44.

33. Steinberg BE, Silverman HA, Robbiati S, et al. Cytokine-specific neurograms in the sensory vagus nerve. Bioelectromed. 2016;3:7-17.

34. Goehler LE, Gaykema RP, Hansen MK, Anderson K, Maier SF, Watkins LR. Vagal immune-to-brain communication: a visceral chemosensory pathway. Auton Neurosci. 2000;85(1-3):49-59.

35. Wood JD, Alpers DH, Andrews PL. Fundamentals of neurogastroenterology. Gut. 1999;49(Suppl. 2):i6–i16.

36. Sanders KM. Regulation of smooth muscle excitation and contraction. Neurogastroenterol Motil. 2008;20(Suppl. 1):39-53.

37. Stonehouse H, Pieplo AL, De Salvia MA, et al. Interleukins 1 beta and 6 induce functional alteration of rat colonic motility: an in vitro study. Eur J Clin Invest. 2003;33(8):704-712.

38. Zhang L, Hu L, Chen M, Yu B. Exogenous Interleukin-6 facilitated the contraction of the colon in a depression rat model. Dig Dis Sci. 2013;58(8):2187-2196.
56. Erces D, Varga G, Fazekas B, et al. N-methyl-D-aspartate receptor antagonist therapy suppresses colon motility and inflammatory activation six days after the onset of experimental colitis in rats. *Eur J Pharmacol*. 2012;691(1-3):225-234.

57. Chang XW, Qin Y, Jin Z, et al. Interleukin-6 (IL-6) mediated the increased contraction of distal colon in streptozotocin-induced diabetes in rats via IL-6 receptor pathway. *Int J Clin Exp Pathol*. 2015;8(5):4514-4524.

58. Athauda D, Foltynie T. The glucagon-like peptide 1 (GLP) receptor as a therapeutic target in parkinson’s disease: mechanisms of action. *Drug Discov Today*. 2016;21(5):802-818.

59. Kissow H, Hartmann B, Holst JJ, Poulsen SS. Glucagon-like peptide-1 as a treatment for chemotherapy-induced mucositis. *Gut*. 2013;62(12):1724-1733.

60. Lebherz C, Kahles F, Piotrowski K, et al. Interleukin-6 predicts inflammation-induced increase of Glucagon-like peptide-1 in humans in response to cardiac surgery with association to parameters of glucose metabolism. *Cardiovasc Diabetol*. 2016;15:21.

61. Li ZY, Zhang N, Wen S, et al. Decreased glucagon-like peptide-1 correlates with abdominal pain in patients with constipation-predominant irritable bowel syndrome. *Clin Res Hepatol Gastroenterol*. 2017;41(4):459-465.

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