Luminal Signaling to Colonic Afferent Nerves

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DISCLOSURE

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OBJECTIVES

• Extrinsic innervation of afferent nerves in the colon

• Epithelial mediators that can activate extrinsic afferents

• Luminal factors that can stimulate release of epithelial mediators to activate afferent nerves

• Describe a lumen to nerve inhibitory pathway
Sensory nerves follow three main pathways from the gastrointestinal tract to the central nervous system—the vagal, splanchnic, and pelvic nerves. Vagal afferent fibres have neuronal cell bodies in the nodose and jugular ganglia, whilst the splanchnic and pelvic innervations have cell bodies in spinal thoracolumbar and lumbosacral dorsal root ganglia (DRGs), respectively. Gastrointestinal afferents in the vagal pathway (tension and mucosal) are typically associated with sensations such as satiety and nausea, although a subpopulation of oesophageal vagal afferents responds at high thresholds, implicating them in pain.

Spinal pathways innervate all viscera and are associated with sensations of pain, discomfort, bloating, and urgency to void. Correspondingly, the majority of vagal afferents innervating smooth muscle respond over a restricted range of distension pressures, whereas spinal afferents respond over a wide dynamic range.

There are differences within the spinal innervation, such that the pelvic pathway contains both non-nociceptive and nociceptive afferents, whilst splanchnic afferents have generally higher mechanical thresholds, with few mucosal and muscular afferents, constituting primarily a nociceptive pathway.

Understanding the specialised roles of TRP channels in visceral pain will depend on determining which of the afferent subtypes and pathways express particular channels, and the consequence of blocking their function in each subtype.

ROLES OF TRP CHANNELS IN SENSORY SIGNALLING

The TRP family comprises five subfamilies (TRPA, TRPC, TRPM, TRPP, and TRPV) in mammals, which share several key properties: they are non-selective cation channels and most have six transmembrane domains (fig 2). As their name suggests, they were first identified as channels mediating brief excitatory events in non-mammalian sensory systems, although their roles have outgrown this original label as evident from the discussion below. They are grouped according to their structural similarities, and are discussed below in order of the interest that has been expressed in them in visceral sensory pathways. For a more complete review of their functions in various physiological systems, see reviews by Clapham, Dhaka et al, and Christensen and Corey.

TRP channels have received fame and/or notoriety in a number of fields, including pain, respiratory and cardiac function, ion metabolism, and absorption.
EXTRINSIC AFFERENTS IN THE COLON

COLONIC ENTEROENDOCRINE CELLS

COLONIC ENTEROENDOCRINE CELLS

- Enteroendocrine cells in the colon contain a number of different mediators

- Predominant mediators include:
  - GLP-1 (L cells)
  - Peptide YY (L cells)
  - 5-HT (Enterochromaffin cells)

- Other mediators
  - Chromogranin A
  - Somatostatin
  - Oxyntomodulin
FUNCTIONAL GI DISORDERS

Mann

5-HT-positive cell count was significantly increased in patients with IBS as compared with HC.

Mucosal Biopsy

IBS patients showed a significant increase in the count of 5-HT-positive cells, as compared with HC.

5-HT immunolabelling (arrows) either in patients with irritable bowel syndrome (IBS) or in healthy controls (HC).

The area of crypt epithelium occupied by 5-HT-positive enterochromaffin cells was significantly greater in diarrhea-predominant IBS (IBS-D) in comparison with constipation-predominant IBS (IBS-C).

The effect of mucosal 5-HT on sensory afferent pathways was studied.

Correlation of EC Cells, 5-HT release, and mast cells with symptoms, including bowel habit.

5-HT release correlated with abdominal pain severity.

5-HT release was not different in the diarrhea- and constipation-predominant subgroups (IBS-D vs. IBS-C).

Our previous data demonstrated that mucosal supernatants from patients with IBS evoked a significantly positive correlation with abdominal pain severity.

Therefore, in this study we focused on the effect of mucosal 5-HT on sensory afferent pathways.
5-HT RESPONSIVE FIBRES FROM EACH GROUP (FIGURE 4)

AS DUNNET’S LEVELS IN ANIMALS AFTER RECOVERY HAD RETURNED TO BASAL LEVELS OF 5-HT IN CONTROLS COMPARED WITH 14 OUT OF 16.

RESULTS SHOWED A SIGNIFICANT INCREASE FROM 2.34 × 10^{-3} TO 4.56 × 10^{-3} U MG^{-1}.

AN ESTIMATED 50% INCREASE IN INFLAMED ANIMALS AT DAY 7.

EFFECTIVE, EVOKED ON APPLICATION OF 5-HT (10^{-5} M), WHICH IS A VERY POTENT ACTIVATOR OF THESE FIBRES.

THE RESPONSE AT MAXIMUM CONCENTRATION, TO 5-HT WAS REDUCED FROM 3.2 × 10^{-3} TO 8.2 × 10^{-3} m.

IT WAS POSSIBLE TO INVESTIGATE WHETHER OTHER ASP...
COLONIC 5-HT RELEASE

• 5-HT release is increased in IBS patients.

• 5-HT can activate colonic afferents mainly via 5-HT$_3$ receptors.

• Following recovery from inflammation the response of afferent fibers to 5-HT is increased.

What luminal factors could stimulate release of 5-HT from epithelium?
NUTRIENT SENSING IN THE COLON

- Food is a common trigger for a large proportion of IBS patients
- Recent clinical studies highlight role of diet modification in treating IBS
- Can nutrients signal to colonic afferent nerves?

Amino Acid Receptors

Fatty Acid Receptors

Symonds, Peiris et al Gut 64:618-26
NUTRIENT SENSING IN THE COLON

A

CaSR

5-HT

Co-label

vii

viii

ix

10μm

B

GLP-1 conc. (ng/mL) per biopsy (g)

PYY conc. (ng/mL) per biopsy (g)

5-HT conc. (ng/mL) per biopsy (g)

Control

LA

Control

LA

LA

Control

*
NUTRIENT-NEURAL SIGNALING IN THE COLON

Lauric Acid 25mM

Spikes (5s)^{-1}
NUTRIENT-NEURAL SIGNALING IN THE COLON

Effect of Antagonists to 5-HT3/Y2/GLP-1 Receptors

A

B

Lauric Acid

Lauric Acid + Antagonists

% 1st Response

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NUTRIENT-NEURAL SIGNALING IN THE COLON

Is the pathway altered in IBS?
BILE ACID SIGNALING IN THE COLON

• ~95% of bile acids absorbed in ileum

• A subpopulation of IBS patients have increased bile acids in the colon
  • Proportion of patients varies depending on method

• Most common side effect of bile acid transport inhibitors for constipation is abdominal pain

Do bile acids signal to colonic afferent nerves?

Bajor et al Gut 64:84-92
Wong et al Am J Gastroenterol 106:2154-64
BILE ACID SIGNALING IN THE COLON

A Rat Distal Colon

B Human Biopsy

C Mouse DRG Neuron

D Mouse Mucosa

Lynn and Blackshaw J Physiol. 518:271-82
Symonds, Peiris et al Gut 64:618-26
Alemi et al J Clin Invest 123:1513-30
Alemi et al Gastroenterology 144:145-54
BILE ACID SIGNALING IN THE COLON

Deoxycholic Acid (DCA) – TGR5 Agonist

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[Diagram showing pressure transducer and spikes/s/mm Hg/µV over time]
BILE ACID SIGNALING IN THE COLON

Deoxycholic Acid (DCA) – TGR5 Agonist

(A) % Units Activated by DCA

(B) Normalized afferent discharge (%)

Pressure mm Hg
Bile and bile acids can activate colonic afferents in the proximal and distal colon.

Activation may be direct (i.e. activation of nerve terminal) or indirect via release of mediator.

Excess bile acids may contribute to abdominal pain in subpopulation of IBS patients.
LUMINAL INHIBITORY SIGNALING

- Activation of guanylate cyclase C (GC-C) on luminal surface increases chloride secretion
- Linaclotide, GC-C agonist, reduces abdominal pain in patients with IBS-C

Mechanism?
LINACLOTIDE REDUCES NOCICEPTION AND PAIN 1343

Similar mechanism in human afferent nerves?

A

![Graph A: cGMP 1000µM](image)

- Change in mechanosensitivity respect to baseline (spikes/sec)
- Mucosa intact vs. Mucosa removed
- n=12 vs. n=9
- Significance: **p<0.01

B

![Graph B: Linaclotide 100nM](image)

- Change in mechanosensitivity respect to baseline (spikes/sec)
- Mucosa intact vs. Mucosa removed
- n=10 vs. n=6
- Significance: ***p<0.001

Castro et al  Gastroenterology145:1334-46
cGMP INHIBITION OF HUMAN COLONIC AFFERENTS

Control cGMP 500µM

Mesentery Serosa

Spike/10s

Control cGMP Washout
cGMP INHIBITION OF HUMAN COLONIC AFFERENTS

Human Appendix

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**Graphical Representation**

- **30 mmHg**
  - Baseline
  - STa 100 nM
  - Washout

- **40 mmHg**
  - Baseline
  - STa 100 nM
  - Washout

- **60 mmHg**
  - Baseline
  - STa 100 nM
  - Washout

*Note:* n.s. indicates not significant at the P < 0.01 level.

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**Statistical Analysis**

- **Paired t-test:**
  - Tissues from 4 patients

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**Legend**

- Baseline
- STa 100 nM
- Washout
cGMP INHIBITION OF COLONIC AFFERENTS

- GC-C agonists bind to GC-C to increase cGMP.
- cGMP is released extracellularly to reduce mechanosensitive colonic afferents signaling.
- GC-C agonists cause greater inhibition than exogenous cGMP.
SUMMARY

- Enteroendocrine cells release mediators (e.g. 5-HT) that can activate extrinsic afferent nerves

- Luminal factors (nutrients, bile acids) can activate colonic afferents, in part by release of enteroendocrine mediators
  
  - Meal induced symptoms in IBS
  - Increase bile acids in IBS patients

- Inhibitory lumen-to-nerve pathway via guanylate cyclase C activation
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