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Gastrointestinal pain in functional bowel disorders: sensory neurons as novel drug targets

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Functional bowel disorders (FBDs) are defined by symptoms of gastrointestinal (GI) dysfunction, discomfort and pain in the absence of a demonstrable organic cause. Since the prevalence of FBDs, particularly functional dyspepsia and irritable bowel syndrome, can be as high as 20%, FBDs represent a significant burden in terms of direct healthcare and productivity costs. There is emerging evidence that the discomfort and pain experienced by many FBD patients is due to persistent hypersensitivity of primary afferent neurons, which may develop in response to infection, inflammation or other insults. This concept identifies vagal and spinal sensory neurons as important targets for novel therapies of GI hyperalgesia. Sensory neuron-specific targets can be grouped into three categories: receptors and sensors at the peripheral nerve terminals, ion channels relevant to nerve excitability and conduction and transmitter receptors. Particular therapeutic potential is attributed to targets that are selectively expressed by afferent neurons, such as the transient receptor potential channel TRPV1, acid-sensing ion channels and tetrodotoxin-resistant Na^+ channels.

Keywords: 5-HT_3 and 5-HT_4 receptors, abdominal pain, acid-sensing ion channels, functional bowel disorders (FBDs), functional dyspepsia, gastrointestinal (GI) hyperalgesia, irritable bowel syndrome (IBS), purinoceptors, sensory neurons, tetrodotoxin-resistant sodium channels, transient receptor potential channel TRPV1

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1. The burden of functional bowel disorders and abdominal pain

Functional bowel disorders (FBDs) such as non-cardiac chest pain, functional (non-ulcer) dyspepsia and irritable bowel syndrome (IBS) are defined by chronic or recurrent abdominal symptom patterns without an organic cause identifiable by conventional diagnostic means [1-3]. Common to all FBDs is that patients suffer from unexplained visceral pain or discomfort, which in IBS is closely linked to a disturbance of bowel habits. The distinct types of FBDs are regarded as separate clinical entities and there is evidence that even functional dyspepsia, as well as IBS, represents a heterogeneous disorder, yet the symptom patterns of IBS can overlap with those of functional dyspepsia and gastro-oesophageal reflux disease (GORD) [3,4]. Whilst psychosocial stressors and gastrointestinal (GI) infections have been recognised as risk factors, the development of FBDs is unpredictable and is characterised by acute episodes interspersed with silent periods or by continuity of long symptomatic periods [1,3].

Although not life-threatening, FBDs cause considerable suffering and can severely impair quality of life. Most relevant from a health economics perspective is that FBDs are very common and account for some 50% of all gastroenterology referrals, despite the fact that only 10 – 50% of adults with symptoms typical of FBDs ever present for medical evaluation [3]. The prevalence of IBS alone has been reported to

range 6.6 – 22% of the adult population in European countries and is higher in women than men [3]. In view of these estimates and the expensive diagnostic workup, the direct and productivity costs associated with the management of IBS place this functional disorder within the ten most expensive GI diseases in the US [5]. The total annual direct costs of IBS management in the UK have been estimated to be £45.6 million and in the US, US$1.35 billion, with the expenditure per patient and year varying between €251 and €823 in European countries, Canada and the US [3,5,6].

2. Gastrointestinal pain mechanisms

2.1 Focus on primary afferent neurons

The pharmacological treatment options for FBDs are limited, which portrays the relative lack of knowledge as to how abdominal pain and hyperalgesia occur. There is now convincing evidence that multiple mechanisms contribute to the initiation and maintenance of FBDs at the level of the GI tract, the afferent nervous system and the brain [1,2,7]. Accordingly, novel therapies of FBDs may be targeted at:

- The derangements of digestive functions.
- The hypersensitivity of afferent neurons.
- The exaggerated processing of afferent information in the brain in the context of a variety of psychosocial factors (gut–brain axis).
- The disturbed control of GI functions by the brain through the autonomic nervous system (ANS) and endocrine mechanisms (brain–gut axis).

Analysis of the possible mechanisms underlying FBDs has shown that abdominal hypersensitivity is an important factor in functional dyspepsia and IBS [1,2,7-11]. Although this functional alteration may occur at peripheral and central levels of the gut–brain axis, it does point at primary afferent neurons as a relevant target for novel therapies. The rational development of drugs directed at hypersensitive afferent neurons requires a thorough exploration of the functional characteristics of GI afferents in health and disease, analysis of the mechanisms whereby they become hypersensitive in disease and identification of molecular targets that are involved in hypersensitivity and are selectively expressed by GI afferents. This paper reviews some of the pertinent advances and discusses sensory neuron-directed approaches that hold potential as novel therapeutics for FBDs.

2.2 Sensory innervation of the gastrointestinal tract

Unlike somatic structures, which are supplied by one population of sensory neurons, the alimentary canal is innervated by two populations of extrinsic afferent neurons [12-14]. Their cell bodies lie either in the jugular and nodose ganglia (vagal afferents) or in the dorsal root ganglia (DRG; spinal afferents). Importantly, 80 – 90% of the axons in the vagus nerves are afferent nerve fibres that project to the nucleus tractus solitarii in the brainstem, whilst only a 10 – 15% minority of the somata in the DRG supplies visceral tissues [14].

The spinal afferent nerve fibres are organised in a segmental manner but, unlike those of somatic afferents, are distributed over several spinal segments. This diffuse termination pattern in the spinal cord explains the diffuse localisation of visceral sensations, and the convergence of visceral and somatic afferents in the spinal cord is thought to account for the referral of visceral pain to segment-equivalent somatic structures [14]. Except for particular spatial arrangements in the myenteric plexus and GI muscle [15], the visceral endings of the vagal and spinal afferents have no end organs or morphological specialisations. Associated mostly with non-myelinated and some thinly myelinated axons, they innervate mucosa, submucosa (particularly arterioles), muscle, myenteric plexus and serosa [14,15]. With these projections and their sensory modalities, they can respond to changes in the chemical environment in the lumen, interstitial space and vasculature and to mechanical distortion of the gut wall, typically distension but also contraction or relaxation of the muscle [14,15].

2.3 Mechanisms of gastrointestinal hyperalgesia

2.3.1 Gastrointestinal sensation in health and disease

The complex tasks of the GI tract to digest food, absorb nutrients and water, eliminate useless material and recognise harmful food constituents, antigens and pathogens, require a differential analysis of the luminal contents so that appropriate effector programmes can be selected [14]. To this end, the digestive system is endowed with an elaborate network of surveillance systems that comprise sensory neurons, enteroendocrine cells and immune cells. With input from the other detector systems, afferent neurons convey information to the enteric nervous system, the CNS and the ANS to regulate digestion and, in the face of harmful conditions, to initiate homeostatic reactions [13,14]. Although there is a continuous flow of information from the gut to the CNS, this input is normally processed only in autonomic and neuroendocrine circuits but does not reach the level of consciousness [16,17]. Abdominal pain is thus a sensation whose pathophysiological meaning is not immediately clear. Whilst, for instance, epigastric symptoms in functional dyspepsia may be a warning sign [18] to abstain from further food intake or to avoid certain types of food, GI pain in most other cases cannot be adequately interpreted with regard to its cause and consequences.

The pain of FBD patients may reflect pathological alterations in gut function and/or signify that events in the GI tract are represented in the brain in an exaggerated fashion because the sensory threshold of afferent neurons is lowered or the central gain of information from the GI tract is enhanced [14]. An association of functional dyspepsia and IBS with GI hypersensitivity has been confirmed in many clinical studies in which sensations evoked by both physiological stimuli such as a meal [19,20] and pathological stimuli such as noxious distension [1,2,7-11,13,14] were found to be amplified. Patients

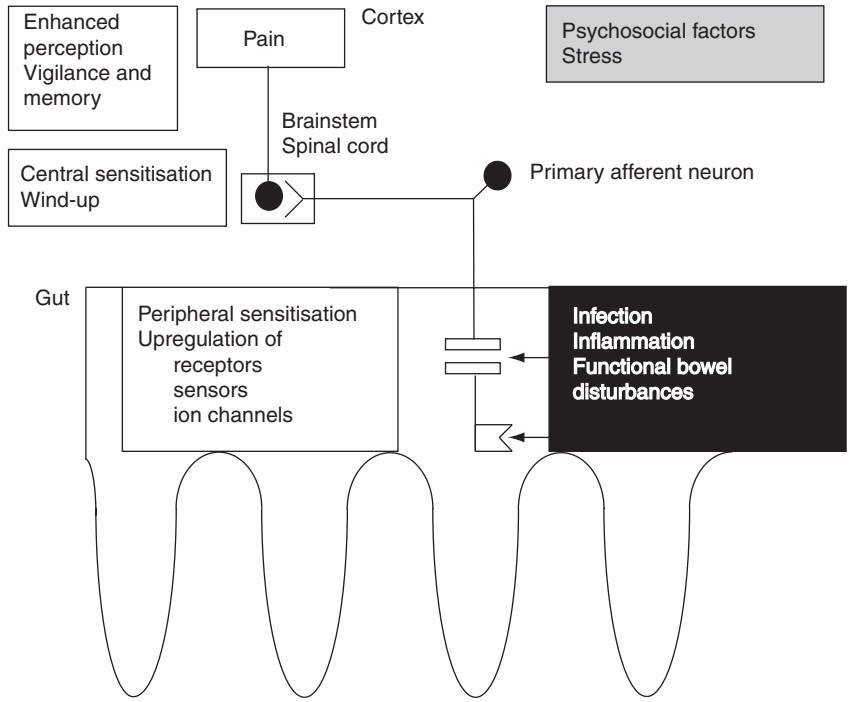

Figure 1. Mechanisms underlying visceral hyperalgesia in FBDs.
FBD: Functional bowel disorder.

with functional dyspepsia are also hypersensitive to chemical stimuli such as fat and acid, although gastric acid secretion is in the normal range [14]. It should not go unnoticed that acid also contributes to gastric and duodenal ulcer pain and that GORD is associated with an enhanced oesophageal sensitivity to acid but not distension [21].

2.3.2 *Gastrointestinal hypersensitivity associated with functional bowel disorders*

Infectious gastroenteritis, which may have subsided long ago, is a risk factor for both functional dyspepsia [22] and IBS [2,23]. It is thus probable that immunological and inflammatory processes can initiate long-lasting changes in bowel function and nociceptive afferent pathways (**Figure 1**). Indirect support for this hypothesis comes from the observations that the number of immunocompetent cells is enhanced in the colon of IBS patients [24] and that there is hypermastocytosis in the gastric and colonic mucosa of patients with functional dyspepsia and IBS, respectively [25,26]. Studies in the rat and mouse demonstrate that gastric and colonic inflammation amplify visceral mechanonociception as revealed by an exaggerated visceromotor response to distension [9,27-30]. Such experimentally induced states of hypersensitivity can become permanent if the priming insult (e.g., irritation of the colon or maternal separation) is experienced early in life [31,32]. In addition to mechanonociception [30], acid-induced chemonociception in the rat stomach is also enhanced following inflammation or ulceration [33].

Helicobacter pylori-induced gastritis in mice leads to upregulation of substance P and calcitonin gene-related peptide (CGRP) in spinal afferent neurons supplying the stomach [34]. The relevance of this finding to functional dyspepsia is not clear because clinical studies do not unequivocally show a benefit of *H. pylori* eradication in the treatment of functional dyspepsia [35]. There is evidence, however, that *H. pylori* infection is associated with an enhanced risk of developing dyspeptic symptoms [36].

2.3.3 *Mechanisms of sensory neuron hypersensitivity in functional bowel disorders*

GI hyperalgesia in FBDs may arise from changes at many levels of the gut–brain axis (**Figure 1**). The concept that primary afferents are a relevant target for treating abdominal pain implies that these neurons are sensitised in states of hyperalgesia or undergo other functional changes that are relevant to hypersensitivity. Indeed, most extrinsic afferents innervating the gut have the ability to sensitise in response to a number of pro-inflammatory mediators and display enhanced excitability following experimentally induced inflammation [37-42]. The mechanisms whereby hypersensitivity and hyperexcitability of afferent neurons are initiated and maintained are thus of prime pharmacological interest, if therapeutic options to prevent or reverse sensitisation are pursued. In analysing the pertinent molecular and cellular processes (**Figure 1**) it is very useful to consider somatic pain mechanisms that have been elucidated

in much more detail and hence provide valuable leads for understanding visceral pain [43].

As long as it is reversible, sensitisation of nociceptors results from modulation of nerve fibre excitability via post-translational changes such as phosphorylation of receptors, ion channels or associated regulatory proteins [43]. In contrast, permanent increases in the sensory gain are related to changes in the expression of transmitters, receptors and ion channels, changes in the subunit composition and biophysical properties of receptors and ion channels or changes in the phenotype, structure, connectivity and survival of afferent neurons. A particular form of sensitisation occurs with a group of afferents that are mechanically insensitive in the healthy tissue but acquire mechanosensitivity after a tissue insult, a process described as awakening of 'sleeping' or 'silent' nociceptors [8]. Experimental studies suggest that neurotrophins and cytokines, which are generated in the inflamed gut, are important factors for the long-term sensitisation of primary sensory neurons [43-45]. Why sensitisation is maintained long after the inflammatory insult has gone is not yet understood. A similar issue relates to the question of why some patients affected with infectious gastroenteritis develop FBDs, whereas others do not. One clue may come from the observations that some patients with IBS exhibit a diminished production of anti-inflammatory cytokines such as IL-10 [46], whilst those patients that develop postinfectious IBS express elevated levels of the pro-inflammatory IL-1β in the colonic mucosa [47].

3. Sensory neurons as targets in the control of gastrointestinal hyperalgesia

3.1 Advantages and disadvantages of sensory neuron-targeting drugs

Although the comorbidity of FBDs with depression, anxiety and related disorders [1,2,7,48,49] suggests that GI hyperalgesia involves many disturbances in the gut–brain (**Figure 1**) and brain–gut axis, sensory neurons serve as the first element at which to aim novel therapies to control GI pain [13,38,50]. In addition, drugs that target nociceptive afferent neurons can be configured such that they do not enter the brain and hence are free of adverse effects on CNS functions. Sensory neuron-targeting drugs, however, can also have disadvantages inasmuch as they may interfere with important physiological functions of primary afferents relevant to digestion and with the regulatory roles of peripheral neurons of the enteric nervous system and ANS. Furthermore, they will be ineffective if hyperalgesia is solely the result of central sensitisation processes.

3.2 Key questions in the design of efficacious sensory neuron-targeting drugs

Ideally, sensory neuron-targeting drugs should block the exaggerated signalling of hypersensitive afferents, which implies that they aim towards molecular targets that are altered in GI disease [13]. Without doubt, the complex innervation of the GI tract complicates the search for specific traits on nociceptive afferents supplying the gut. In exploiting such molecular targets, it is important to address several key questions that are crucial to the development of an efficacious and safe visceral analgesic:

- Which mechanical and chemical stimuli in the gut, noxious or innocuous, are relevant to GI discomfort and pain?
- Which receptors and ion channels on extrinsic afferents are relevant to the exaggerated gut–brain signalling in FBDs and other conditions of abdominal hyperalgesia?
- Which extrinsic afferents (vagal or spinal) contribute to GI discomfort and pain? Are different stimulus modalities signalled by anatomically and neurochemically distinct populations of sensory neurons?
- Do afferent neurons involved in GI discomfort and pain express receptors, ion channels or other molecular traits that are specific to them and absent from other peripheral neurons?
- Is the expression of sensory neuron-specific molecular targets, which are relevant to nerve function, altered in states of abdominal hypersensitivity?
- Is drug interference with molecular targets on GI afferents, which are thought to be disease-relevant, efficacious and safe in the treatment of GI hyperalgesia?

3.3 Three classes of sensory neuron-targeting drugs

Many efforts in the current search for new treatments of abdominal pain are directed at primary afferent neurons [13,14,38]. In broad terms, sensory neuron-specific targets can be grouped into three categories (**Box 1**):

- Receptors and sensors at the peripheral terminals of afferent neurons that are relevant to stimulus sensitivity.
- Ion channels that govern the excitability and conduction properties of afferent neurons.
- Transmitters and transmitter receptors that mediate communication between primary afferents and second-order neurons in the spinal cord and brainstem.

The category of receptors and sensors on afferent nerve terminals comprises a large number of targets, as listed in **Box 1**. Of note is a family of more than 50 orphan G-protein-coupled receptors, termed Mrgs, which are expressed in specific subsets of afferent neurons known to detect painful stimuli [51-53]. It remains to be elucidated as to which stimuli and agonists other than RF-amide-related peptides [52] and proenkephalin A gene products [53] can activate these receptors and whether or not Mrgs are relevant to GI hypersensitivity. Among the ion channels relevant to nerve excitability and conduction (**Box 1**), it is the tetrodotoxin-resistant $Na_v1.8$ sodium channel that has attracted most attention.

Primary sensory neurons can be differentiated by their chemical coding in terms of transmitter expression, with glutamate, CGRP and the tachykinins substance P and

Box 1. Three classes of drug target on sensory neurons.

Receptors and sensors on afferent nerve terminals
5-HT_3 and 5-HT_4 receptors
Adenosine A_1 and A_2 receptors
Ionotropic $P2X_2$, $P2X_3$ and $P2X_{2/3}$ purinoceptors
Transient receptor potential TRPV1 and TRPV4 receptors
Acid-sensing ion channel ASIC1, ASIC2, ASIC3 and ASIC2b/3 receptors
BK B_1 and B_2 receptors
PG EP_1, EP_3, EP_4 and IP receptors
PAR-1 and PAR-2
Cholecystokinin CCK_1 receptors
Corticotropin-releasing factor receptors
Somatostatin sst_2 receptors
Ionotropic and metabotropic glutamate receptors
μ-, κ- and δ-opioid receptors
Cannabinoid CB_1 receptors
Orphan G-protein-coupled receptors (Mrgs)
Neurotrophin receptors
Mechanosensitive K^+ and Ca^{2+} channels

Ion channels relevant to nerve excitability and conduction
Voltage-gated Ca^{2+} channels
Voltage-gated K^+ channels
Tetrodotoxin-resistant voltage-gated Na^+ channels

Transmitters and transmitter receptors
Ionotropic and metabotropic glutamate receptors
CGRP receptors
Tachykinin NK_1, NK_2 and NK_3 receptors

For details see text and [13,38,50,169,179,181,182].
5-HT: 5-Hydroxytryptamine; BK: Bradykinin; CGRP: Calcitonin gene-related peptide; PAR: Protease-activated receptor; PG: Prostaglandin.

neurokinin A being the prevalent messenger molecules [13,54,55]. As a consequence, antagonists of glutamate [13,55,56], CGRP [27,29] and tachykinin [13,28,57,58] receptors are explored as possible therapeutics for functional dyspepsia and IBS (**Box 1**). This category of sensory neuron-targeting drugs is not further considered in this article.

In assessing the significance of targets on sensory neurons in visceral hyperalgesia it is important to explore whether or not number, subunit composition and biophysical properties of sensory neuron-specific ion channels and receptors are persistently altered in GI disease [13]. Appropriate experimental models of GI disease and clinical proof-of-concept studies are required to critically evaluate the quantitative contribution the sensory neuron-specific targets make to the induction and/or maintenance of GI hyperalgesia and whether or not modulation of a single target is therapeutically sufficient. With these considerations in mind, the following sections of this article will discuss a select group of sensory neuron-specific receptors, sensors and ion channels that have potential in the therapy of visceral hypersensitivity and are currently the focus of interest.

4. Sensory neuron-specific receptors and sensors

4.1 5-HT_3 and 5-HT_4 receptors

Many efforts to develop novel drugs for FBDs have been directed at 5-hydroxytryptamine (5-HT) receptors, with mixed results. 5-HT is an interesting target because the enterochromaffin cells of the gut represent the major source of 5-HT in the body. Released by a variety of luminal stimuli, 5-HT can activate intrinsic and extrinsic sensory nerve fibres as well as other types of enteric neurons through activation of multiple 5-HT receptors [50,59,60]. Most research has been focused on 5-HT_3 and 5-HT_4 receptors in an attempt to correct both the functional disturbances in the gut and the pain associated with FBDs. Although 5-HT_3 and 5-HT_4 receptors are not confined to primary afferents but are also expressed by enteric neurons and other cells of the gut, 5-HT-evoked excitation of extrinsic sensory neurons is primarily mediated by 5-HT_3 receptors [38,50,59]. A role of 5-HT in FBDs is also suggested by the observations that IBS can be associated with changes in colonic 5-HT levels and alterations in the serotonin re-uptake transporter that governs the availability of 5-HT at its receptors [61,62].

Antagonism of 5-HT_3 receptor-mediated stimulation of vagal afferents inhibits emesis induced by release of 5-HT from enterochromaffin cells [50], whereas blockade of 5-HT_3 receptor-mediated activation of spinal afferents by alosetron depresses the afferent signalling of colorectal distension in the rat [63]. Accordingly, alosetron has been found to reduce the discomfort and pain experienced by female patients suffering from functional dyspepsia or diarrhoea-predominant IBS to a moderate but significant extent [59,60,64]. In view of the non-selective distribution of 5-HT_3 receptors to extrinsic sensory neurons, the utility of alosetron is limited by its inhibitory action on intestinal peristalsis and fluid secretion resulting in constipation [59,64]. In addition, the use of alosetron has been severely restricted by its effect to increase the incidence of ischaemic colitis in IBS patients [59,64]. It remains to be seen as to whether or not other 5-HT_3 receptor antagonists in development, such as cilansetron, will fare better in this respect.

5-HT_4 receptor agonists such as cisapride have been in use to stimulate foregut motility, and the partial 5-HT_4 receptor agonist tegaserod has been licensed for the treatment of constipation-predominant IBS. Whilst stimulating colonic transit, tegaserod also seems to reduce pain and other symptoms in female patients with constipation-predominant IBS [64-66] and to attenuate the pain evoked by rectal distension in healthy subjects [67]. The moderate clinical efficacy of tegaserod is in line with experimental studies in which this drug has been found to inhibit the afferent signalling of colorectal distension in the rat and cat, particularly if there is inflammation in the colon [68,69]. The precise mechanism and site of action whereby tegaserod is antinociceptive remains to be identified.

4.2 Prostaglandin receptors

Inflammation induces the synthesis of large quantities of prostaglandins (PGs) through COX-2, and PGs such as PGE_2 and PGI_2 are key mediators of inflammatory hyperalgesia. Whilst suppression of PG production in the gut by COX inhibitors carries the risk of severe GI mucosal damage, blockade of PG receptors expressed by sensory neurons may seem a more favourable and selective way of preventing the pro-algesic action of PGs. Indeed, primary sensory neurons express PG receptors of the EP_1, EP_2, EP_{3C}, EP_4 and IP type [70,71], and PGE_2 excites mesenteric afferent nerve fibres supplying the rat jejunum by a direct action on neuronal EP_1 receptors [72].

Apart from activating sensory neurons, PGs sensitise abdominal afferents to other algesic chemicals such as bradykinin (BK) [73]. Experiments with DRG neurons in culture indicate that both EP_{3C} and EP_4 receptors contribute to the PGE_2-induced sensitisation of sensory neurons [71]. Likewise, EP_3 and IP receptors participate in the endotoxin-evoked sensitisation of peritoneal afferents in mice, as assessed by the writhing response to intraperitoneal acetic acid [74]. The acid-induced sensitisation of the human oesophagus to electrically induced pain is attenuated by the EP_1 receptor antagonist ZD-6416 [75]. However, the implication of PG receptors in experimental models of functional dyspepsia and IBS has not yet been explored.

4.3 Protease-activated receptors

Protease-activated receptors (PARs) of type PAR-1 and -2 are expressed by DRG neurons containing CGRP [76,77]. Accordingly, PAR-2 agonists are able to excite spinal afferents in rat jejunal mesenteric nerves [78] and to release CGRP from DRG neurons in culture [77]. Likewise, intracolonic or intrapancreatic administration of a PAR-2 agonist elicits afferent input to the spinal cord, as visualised by c-Fos expression [77,79]. Activation of PARs, however, not only causes acute stimulation of sensory neurons but also gives rise to prolonged hyperalgesia. Thus, stimulation of mucosal PAR-2 in the rat colon brings about a delayed hypersensitivity to colorectal distension [79] and administration of a PAR-2 agonist into the pancreatic duct sensitises spinal afferents to the excitatory effect of capsaicin [77].

From these findings it would appear that PAR-2 antagonists have potential in the control of visceral pain and hyperalgesia. In addition, they may have anti-inflammatory activity, given that the levels of the PAR-2 agonists trypsin and mast cell tryptase are elevated in the colon of inflammatory bowel disease (IBD) patients and administration of PAR-2 agonists into the mouse colon induces inflammation via a neurogenic mechanism involving sensory neurons [80,81]. Furthermore, exposure of the mouse colon to a PAR-2 agonist enhances the expression of PAR-2 mRNA [80], much as the expression of PAR-2 on colonic mast cells is upregulated in ulcerative colitis [82]. It is not yet known how the pro-inflammatory and pro-algesic effects of PAR-2 activation are interrelated and it is at present difficult to say whether or not PAR-2 antagonists are useful therapeutics for GI hyperalgesia, given that PARs are also present on enteric neurons and GI effector cells and play a role in normal digestive functions [83,84].

4.4 Ionotropic purinoceptor ion channels

P2X purinoceptors are ligand-gated membrane cation channels that open when extracellular ATP is bound. They are assembled as homo- or heteromultimers of several subunits, seven of which ($P2X_1$ – $P2X_7$) have been identified at the gene and protein level [85,86]. The P2X receptors on nodose ganglion neurons comprise predominantly homomultimeric $P2X_2$ and some heteromultimeric $P2X_{2/3}$ receptors, whereas on DRG neurons, homomultimeric $P2X_3$ prevail over heteromultimeric $P2X_{2/3}$ receptors [85,87]. Since ATP is released from a number of cellular sources in response to both physiological and pathological stimuli and excites vagal, mesenteric and pelvic afferent neurons of the rat via activation of P2X receptors [88-93], these receptors can be envisaged as potential targets for controlling abdominal sensation. For instance, ATP seems to be relevant to mechanosensory transduction in the colorectum and urinary bladder where ATP released from epithelial cells by distension activates P2X receptors on pelvic afferents and thereby contributes to the reflex regulation of micturition and colorectal function [92-94].

An implication of P2X receptors in GI nociception may be inferred from the observations that (i) following pepsin-induced inflammation of the ferret oesophagus, ATP

sensitises vagal afferents to mechanical stimuli [94], (ii) $P2X_2$ homo- and heteromultimers are sensitised by acidosis [86,96] and (iii) P2X receptors on sensory neurons are upregulated by experimental inflammation [97]. Likewise, IBD is associated with an increase in the number of $P2X_3$ receptors in the colon [98]. Although P2X receptors in the gut are expressed not only by sensory neurons but also by enteric neurons and smooth muscle cells [85,99], there is reason to speculate that P2X receptors contribute to GI pain and that, hence, $P2X_3$ and $P2X_{2/3}$ receptor blockers could be of therapeutic value [88]. However, the failure of A-317491, a non-nucleotide antagonist of $P2X_3$ and $P2X_{2/3}$ receptors, to attenuate the visceromotor response to colonic distension in the rat and to reverse the mechanical hyperalgesia seen after induction of colitis by zymosan [100], argues against an involvement of $P2X_3$ and $P2X_{2/3}$ receptors in GI mechanonociception. The situation is different, however, with chemonociception, since trinitrophenyl-ATP (a $P2X_1$, $P2X_3$ and $P2X_{2/3}$ receptor blocker) and A-317491 are able to suppress the nociceptive behaviour provoked by intraperitoneal injection of acetic acid into mice [100,101]. Antagonists of $P2X_3$ and $P2X_{2/3}$ receptors may thus have therapeutic potential in the treatment of acid-related, and inflammation- and ischaemia-induced disturbances of gut sensation.

4.5 TRPV1

If there is a hot spot in contemporary pain research, it is certainly with the superfamily of TRP (transient receptor potential) ion channels [102,103], particularly the 'capsaicin receptor' TRPV1 previously termed vanilloid receptor (VR)1 [104]. TRP channels represent an ancient sensory apparatus of the cell, responding to temperature, touch, osmolarity, pH and various chemical messengers [102]. One of the many remarkable properties of TRP channels is that TRPV1, TRPV2, TRPV3, TRPV4, TRPM8 and ANKTM1 are thermosensors with different working ranges, which enable sensory neurons to monitor a wide spectrum of temperatures from noxious cold to noxious heat [105]. TRPV1 is a non-selective cation channel with high permeability for Ca^{2+}, which behaves as a polymodal nociceptor that is activated not only by noxious heat but also by ligands containing a vanillyl moiety such as capsaicin and resiniferatoxin, H^+ ions, ethanol and a variety of arachidonic acid-derived lipid mediators [96,102,106-110].

Importantly, TRPV1 may be a central factor in hyperalgesia because its activity is regulated by many pro-algesic pathways. Thus, activation of PGE_2, BK B_2 and P2Y purine receptors can sensitise TRPV1 through phosphorylation of the channel [111] or other mechanisms [112], and thereby enhance the probability of channel gating by heat and other stimuli [107-109]. Mild acidosis (pH 7 – 6) likewise sensitises TRPV1, whereas a fall of the extracellular pH to < 6 directly gates the channel [106]. A common result of these sensitisation processes is that the temperature threshold for TRPV1 activation (43°C) is lowered to a level permissive for channel gating at normal body temperature [113]. The relevance of TRPV1 to inflammatory hyperalgesia is borne out by the finding that TRPV1 knockout mice do not develop thermal hyperalgesia in response to experimental inflammation [114,115].

A large number of pharmacological studies indicate that, in the gut, TRPV1 is exclusively associated with primary afferent neurons [116]. This inference has been proved by immunohistochemical studies in the rat, guinea-pig and mouse GI tract, in which numerous TRPV1-positive nerve fibres occur in the musculature, enteric nerve plexuses and mucosa [117,118]. Since enteric neurons do not stain for TRPV1, it follows that the TRPV1-positive nerve fibres in the intestine represent processes of spinal afferents and, in the stomach, of some vagal afferents [117,118]. It remains to be elucidated as to whether or not the TRPV1-like immunoreactivity that other investigators have seen in guinea-pig, porcine and human enteric neurons [119-121] and rat gastric epithelial cells [122] represents authentic TRPV1.

Capsaicin-induced gating of TRPV1 stimulates extrinsic afferents of the gut [73,123,124] and gives rise to GI pain in humans [125-127] and mice [128]. Although experimental paradigms of GI hyperalgesia have not yet been explored in TRPV1-deficient mice, there is indirect evidence that TRPV1 contributes to the sensitisation of GI afferent neurons. This inference is based on the ability of capsaicin to induce a state of sensory refractoriness [129], which is associated with a downregulation of TRPV1 [130]. Such a state of functional desensitisation can be achieved by systemic administration of high doses of capsaicin to experimental animals or by repeated topical administration of moderate doses of capsaicin to humans. Capsaicin pretreatment of rats blocks the visceromotor response to gastric acid challenge [33], suppresses the cardiovascular pain response to noxious jejunal distension in the rat [131] and prevents inflammation-induced hypersensitivity to colonic distension [27,132]. Chronic administration of capsaicin is also beneficial in patients experiencing GI pain. Thus, intractable idiopathic pruritus ani can be relieved by a 4-week treatment course with topical capsaicin [133], and daily intragastric administration of red pepper containing 1.75 mg capsaicin for 5 weeks significantly reduces epigastric pain and other symptoms of functional dyspepsia [134]. However, in the initial phase of red pepper administration, when capsaicin is still stimulating afferent neurons, there seems to be an exacerbation of dyspeptic and IBS symptoms [134,135]. Obviously, the initial pungency of a TRPV1 agonist could be avoided by the use of TRPV1 antagonists.

Consistent with a role in GI pain and hyperalgesia is that TRPV1-like immunoreactivity on submucosal nerve fibres in the colon is amplified in patients with painful IBD [136]. Rectal hypersensitivity and faecal urgency are likewise associated with an increase of TRPV1-positive nerve fibres in the muscle, submucosa and mucosa of the rectum and of TRPV1-positive neurons in the myenteric and submucosal plexus [121]. This upregulation of TRPV1 in GI disease is in keeping with experimental observations that inflammation enhances TRPV1 expression and function, a process in which

Box 2. TRPV1 channel blockers.

Capsazepine [183]
5-Iodo-resiniferatoxin [184]
N-alkyl glycine trimers [185]
N-(3-acyloxy-2-benzylpropyl)-N′-[4-(methylsulfonylamino)benzyl]thiourea vanilloid analogues [186,187]
Non-vanilloid SC-0030 [188]
4-(2-Pyridyl)piperazine-1-carboxamides [189]
Cinnamide SB-366791 [190]
7-Hydroxynaphthalen-1-yl-urea and -amide compounds [191]

nerve growth factor (NGF) plays a particular role [137]. From a therapeutic perspective, therefore, TRPV1 antagonists appear to be of great value in suppressing GI hyperalgesia related to FBDs. The search is on and several new TRPV1 blockers have been published in the last 2 years (**Box 2**). Apart from being antihyperalgesic, these drugs may also have anti-inflammatory activity, given that, in rats, TRPV1 is involved in the ileitis evoked by *Clostridium difficile* toxin A [138] and in the colitis elicited by dextrane sulfate [139]. The utility of TRPV1 blockers has yet to be ascertained in established paradigms of GI hyperalgesia and pain. In these tests it will also be important to explore whether or not blockade of TRPV1 interferes with the physiological function of TRPV1-expressing neurons in GI mucosal homeostasis [116].

4.6 Acid-sensing ion channels

Acid-sensing ion channels (ASICs) are members of the voltage-insensitive, amiloride-sensitive epithelial Na^+ channel/degenerin family of cation channels [96,140-142]. They are encoded by four different genes: ASIC1, ASIC2, ASIC3 and ASIC4, with ASIC1 and ASIC2 each having alternative splice variants termed ASIC1a and ASIC1b as well as ASIC2a and ASIC2b. Functional channels are made up of different ASIC subunits, most of which are expressed by primary afferent neurons, although to varying degrees [143,144]. Importantly, ASIC2b, which is inactive as a homomultimer, can form functional heteromultimers with other ASIC subunits, particularly ASIC3, which is exclusively expressed by small and large DRG cells [140,143-145] and, for this reason, is also termed DRASIC.

As their name implies, ASIC1, ASIC2 and ASIC3 are gated by a drop in the external pH to < 6.9 [96,140-142]. In addition, ASICs are mechanoreceptors [142,146] and studies involving deletion of the ASIC2 and ASIC3 genes point to a role of these channels in the transduction of low and high threshold mechanosensation in the skin, respectively [147-149]. Although an implication in abdominal pain has remained unexplored, ASICs could conceivably play a role in the transduction of pressure, distension and acidosis in the GI tract [96]. Since the colonic expression of ASIC3, but not ASIC1 and ASIC2, is upregulated in IBD [150], and since NGF as well as pro-inflammatory mediators such as 5-HT, IL-1 and BK can promote the transcription of ASIC3 in sensory neurons [151], it is tempting to speculate that ASICs contribute to GI inflammatory hyperalgesia. This hypothesis is yet to be tested by genetic and pharmacological approaches.

4.7 Mechanosensitive ion channels

Despite the fact that GI hypersensitivity in FBDs and in experimental models of hyperalgesia is probed almost exclusively by the perceptions and reactions to distension, the molecular sensors of noxious GI distension are largely unknown. Low- and high-threshold mechanosensitive afferents innervate all regions of the alimentary canal and have the ability to sensitise in response to inflammatory mediators [8,152]. Their mechanosensitivity depends on the presence of sensors that detect stretch, contraction or other mechanical deformations of the gut wall. One of these sensors, a mechanosensitive K^+ channel, has been characterised by single-channel recordings from sensory neurons in the rat colon [153]. DRG neurons innervating the stomach and colon exhibit stretch-sensitive calcium fluxes that are inhibited by gadolinium, a blocker of mechanosensitive ion channels [154]. Other mechanosensitive ion channels comprise ASIC1, ASIC2, ASIC3 [146-149], TRPV4 [155] and members of the tandem-pore K^+ channels such as TREK-2 [156], but it awaits to be explored as to whether or not they play a role in GI mechanonociception. If blockers of mechanosensitive ion channels are envisaged as GI analgesics, their possible interference with mechanically triggered motor and secretory reflexes regulating digestion will have to be taken into account.

5. Ion channels regulating sensory nerve excitability, conduction and transmission

5.1 Sensory neuron-specific Na^+ channels

Voltage-gated Na^+ channels, composed of one pore-forming α-subunit and one or more auxiliary β-subunits, are crucial for neuronal excitability and propagation of action potentials. Among the ten known α-subunits are two tetrodotoxin-resistant Na^+ channels, $Na_v1.8$ (previously termed SNS/PN3) and $Na_v1.9$ (SNS2/NaN), and one tetrodotoxin-sensitive Na^+ channel, $Na_v1.7$ (PN1), which are mainly expressed by primary afferent neurons [157,158]. Further analysis has shown that the $Na_v1.7$, $Na_v1.8$ and $Na_v1.9$ subunits are preferentially distributed to DRG neurons with nociceptive properties [159-161].

Tetrodotoxin-resistant Na^+ currents are also present in vagal and spinal afferent neurons supplying the rat stomach [39,40,44] and in DRG neurons projecting to the rat ileum and colon [41,123,162,163]. The tetrodotoxin-resistant currents of the colonic afferents have the characteristics of those carried by the $Na_v1.8$ subunit [162].

A body of evidence obtained from studies of somatic pain shows that the $Na_v1.8$ and $Na_v1.9$ subunits play a role in neuropathic and inflammatory hyperalgesia [157,158]. There is mounting evidence that tetrodotoxin-resistant Na^+ channels also contribute to visceral pain. Experimental gastritis [40], gastric ulceration [39] and trinitrobenzene sulphonic acid-induced ileitis [41,163] enhance the excitability of DRG neurons innervating the respective region of the GI tract, a change that is mainly due to an increase in the tetrodotoxin-resistant Na^+ currents. Similar alterations in vagal afferents are seen in rats with acetic acid-induced gastric ulcers [39]. The upregulation of tetrodotoxin-resistant Na^+ currents in DRG neurons following GI inflammation and injury is likely to involve NGF and pro-inflammatory mediators such as PGE_2 [44,162].

Evidence for a specific contribution of $Na_v1.8$ to visceral hyperalgesia has come from experiments with antisense probes and knockout mice. Thus, antisense probe-induced inhibition of $Na_v1.8$ expression in rat spinal afferents prevents the effect of intravesical acetic acid to induce bladder hyperactivity, a model indicative of bladder hyperalgesia [164]. Null mutation of the $Na_v1.8$ gene does not alter behavioural pain responses to acute noxious stimulation of abdominal viscera but attenuates behavioural reactions to intracolonic administration of capsaicin or mustard oil and prevents referred hyperalgesia [165]. These observations are thought to reflect an implication of $Na_v1.8$ channels in the ongoing activity of colonic afferents sensitised by capsaicin or mustard oil. Taking all findings together, it would seem that tetrodotoxin-resistant Na^+ channels, particularly $Na_v1.8$, constitute a new target for the treatment of visceral hyperalgesia due to inflammation. Although no selective blockers for tetrodotoxin-resistant Na^+ channels are yet available, non-selective inhibitors of voltage-gated Na^+ channels such as lidocaine [166], mexiletine and carbamazepine [167] suppress the central signalling of colonic distension by spinal afferents. It has been suggested that the analgesic effect of the antidepressant drug amitryptiline may also arise from a use-dependent block of voltage-dependent Na^+ channels on sensory neurons [168].

5.2 Sensory neuron-specific K^+ channels

The excitability of sensory neurons is influenced by voltage-gated potassium (K_v) channels such that a downregulation of these channels results in hyperexcitability [169]. This type of change has been found in nociceptive DRG neurons innervating the guinea-pig ileum affected by trinitrobenzene sulphonic acid-induced inflammation [41,163]. The hyperexcitability and increase in conduction velocity seen in ileitis can, in part, be attributed to a decrease in both the transient A-type and sustained outward rectifier K^+ current [163]. Acetic acid-induced gastric ulceration leads to a similar rise of excitability and fall of A-type K^+ current density in spinal and vagal afferents innervating the rat stomach [42]. It should not go unnoticed in this context that the type of homotetrameric $K_v1.4$ channels is selectively expressed by nociceptive afferent neurons [170]. Since neuropathic pain is associated with a decrease in $K_v1.4$ channel density [170], the question arises of how $K_v1.4$ channels behave under conditions of abdominal hyperalgesia.

5.3 Sensory neuron-specific Ca^{2+} channels

There is emerging evidence that certain voltage-gated Ca^{2+} channels on sensory neurons may be of relevance to visceral pain [169]. This contention is based on the antinociceptive effect of gabapentin and pregabalin, two anticonvulsant drugs with high affinity for the $\alpha_2\delta$ Ca^{2+} channel subunit in DRG neurons [171,172]. Gabapentin and pregabalin are able to counteract the colonic hyperalgesia elicited by septic shock [173] or inflammation due to trinitrobenzene sulphonic acid [174]. The writhing response to intraperitoneal injection of acetic acid is also inhibited by gabapentin [175]. Since pregabalin does not alter the visceromotor response to distension of the normal colon [174], it is inferred that pregabalin-sensitive Ca^{2+} channels play a specific role in inflammation-evoked sensitisation of GI afferents. Another Ca^{2+} channel targeted by analgesic drugs is the high voltage-gated N-type Ca^{2+} channel, which is of paramount importance for transmitter release. Inhibition of this channel by intrathecal administration of ziconotide affords relief from chronic pain by blocking transmitter release from the central terminals of spinal afferent neurons [176]. In this way, ziconotide also suppresses the spinal transmission of nociceptive information from mesenteric afferents [177].

6. Expert opinion

There is now good reason to assume that abdominal pain associated with FBDs, particularly IBS, involves persistent sensitisation of GI afferent neurons (**Figure 1**). Although central sensitisation processes and distorted processing and representation of the incoming information in the brain are also involved [1,2,7,75], the contribution made by sensory neurons should not be underestimated. It is via these afferents that the discomfort and pain localised to abdominal viscera is signalled to the CNS. Furthermore, visceral sensory neurons are usually polymodal and all of them seem to have the capacity to sensitise [178]. In view of these properties it can be predicted that sensitisation of GI afferents by inflammatory events may tremendously increase the afferent input to the brain [178]. If this state of exaggerated responses to GI stimuli persists after inflammation has subsided, physiological processes in the alimentary canal may be interpreted by the CNS as inappropriately painful [178]. For all of these reasons, GI afferent neurons represent an intriguing target at which to aim novel therapies for GI discomfort and pain.

Efforts to identify molecular traits that are specific for sensory neurons and therefore hold potential for therapeutic exploitation have been remarkably successful (**Box 1**). These targets include, among others, TRPV1, ASICs (ASIC2b/3), tetrodotoxin-resistant Na^+ channels ($Na_v1.8$) and ionotropic purinoceptors ($P2X_{2/3}$ and $P2X_3$). Since many of these sensors and ion channels are selectively expressed by subpopulations of afferent neurons thought to subserve a nociceptive function, drugs directed at those targets may be antinociceptive without necessarily interfering with physiological functions of afferent neurons. Changes in the expression and functional properties of sensory neuron-specific molecules in GI hyperalgesia may add to the selectivity of drugs directed at these molecules. This concept is borne out by observations that blockade of certain sensory neuron-specific targets reverses experimentally induced GI hyperalgesia but does not influence acute nociception. In addition, selectivity for targets on nociceptive afferent neurons, and preferentially to visceral but not somatic afferents, will be a considerable asset for drug safety.

Despite important advances in the identification of sensory neuron-specific drug targets, there are a number of caveats and uncertainties to be considered if these advances are to be translated into the development of efficacious and safe drugs. A number of these uncertainties are related to our still-fragmented understanding of FBD pathogenesis:

- There is no animal equivalent of FBDs, although it is possible to model individual symptoms [2,179].
- The available animal models of GI hyperalgesia are deficient inasmuch as they do not assess pain perception but, by recording cardiovascular or visceromotor responses, measure pseudo-affective autonomic responses to noxious stimuli. Reliable quantitation of pain perception and emotional-affective alterations in animals will require real-time functional brain imaging.
- Most animal models of GI pain (as well as clinical studies) are modality-biased inasmuch as they assess only reactions to mechanical stimuli. It is highly probable that the chemical environment in the gut lumen also contributes to the noxious background in FBDs. Thus, assessment of pain reactions to chemical stimuli, such as acid or capsaicin [33,75,126,127,180], will be important in the development of drugs targeting GI nociceptive afferents.
- GI sensitivity is complicated by the dual sensory innervation of the gut. Vagal afferents make a significant contribution to the sensory innervation of the oesophagus and proximal GI tract, whereas spinal afferents are distributed throughout the gut [181]. There is increasing awareness that vagal afferents contribute to GI nociception, particularly to chemonociception in the foregut [14,33,180]. This aspect has not yet been considered in the preclinical development of FBD drugs.
- Since it is not known how much sensitisation of afferent neurons versus central sensitisation contributes to GI hyperalgesia in FBDs, it is at present difficult to predict how efficacious blockade of sensory neuron-specific receptors and ion channels will be in correcting GI hypersensitivity.
- Owing to the lack of knowledge about which mechanical and chemical stimuli elicit GI pain in FBDs, it is difficult to say whether or not targeting a single receptor or sensor on GI afferents will be sufficient to manage hyperalgesia. It is speculated that the most efficacious approach will be to block polymodal nociceptors such as TRPV1 or ion channels involved in the propagation of hyperalgesia-related signals such as $Na_v1.8$.

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