

## REVIEW ARTICLE

# Sensory neurone responses to mucosal noxae in the upper gut: relevance to mucosal integrity and gastrointestinal pain

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**Abstract** The digestive tract is supplied by extrinsic and intrinsic sensory neurones that, together with endocrine and immune cells, form a surveillance network that is essential to gut function. This article focuses on the responses of extrinsic afferent neurones to chemical insults of the gastrointestinal mucosa and their pathophysiological relevance to mucosal integrity and abdominal pain. Within the gastroduodenal region, spinal afferents subserve an emergency function because, in case of alarm by influxing acid, they stimulate mechanisms of mucosal protection via an efferent-like release of transmitters. Other sensory neurones signal chemical noxae to the brain, a task that is not confined to spinal afferents because vagal afferents communicate gastric acid and peripheral immune challenges to the brainstem and in this way elicit autonomic, endocrine, affective and behavioural reactions. Emerging evidence indicates that hypersensitivity of extrinsic afferent pathways to mechanical and chemical stimuli makes an important contribution to the abdominal hyperalgesia seen in functional dyspepsia and irritable bowel syndrome. Sensitization may be brought about by inflammatory processes that lead to up-regulation and functional alterations of receptors and ion channels on sensory neurones. Such sensory neurone-specific molecules, which include vanilloid (capsaicin) receptors, may

represent important targets for novel drugs to treat abdominal pain.

**Keywords** cytokines, efferent-like function of afferent neurones, gastric mucosal integrity, gastrointestinal chemonociception, mechanisms of hyperalgesia, vanilloid receptors.

## THE GUT IN NEED OF A SENSORY INNERVATION

The gastrointestinal (GI) tract contains the largest collection of nerve cells outside the brain<sup>1</sup> so that, with good reason, the gut can be regarded as a neurological organ.<sup>2</sup> Digestion is under the control of various neural reflex circuits, among which primary afferent neurones take a prominent place. Many sensory neurones can, in addition, recognize potentially or actually harmful conditions (noxae) in the GI lumen and wall. If the structural and functional features of the GI mucosa are taken into account, it can easily be appreciated that the gut is in need of nociceptive monitoring devices. Just consider that the human GI mucosa covers the enormous area of some 300 m<sup>2</sup>, which is in keeping with the digestive and absorptive function of the gut. At the same time, the GI tract needs to eliminate useless material and to recognize harmful food constituents, antigens and pathogens in order to neutralize or expel them via emesis and diarrhoea.<sup>3,4</sup> In the upper gut, even the secretions of the stomach such as acid and pepsin can be deleterious if the self-defence of the mucosa is failing.

These seemingly conflicting tasks of the alimentary canal require a differential analysis of the luminal contents and of the milieu within the GI wall so that

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the appropriate effector programmes can be selected (Fig. 1). To this end, the digestive system is endowed with an elaborate network of surveillance systems that comprise intrinsic and extrinsic sensory neurones as well as specialized epithelial cells and a vast number of immune cells.<sup>3,4</sup> While intrinsic primary afferent neurones (IPANs)<sup>5</sup> supply the enteric nervous system (ENS) with information that this brain of the gut requires for its independent control of digestion, afferent fibres of the vagal and spinal nerves convey information from the gut to the brain.<sup>3,4,6-9</sup> By reporting on the circumstances that are relevant to energy, fluid and electrolyte homeostasis of the body, extrinsic afferents participate in autonomic and neuroendocrine reflex circuits, but the information that they convey to the brain is rarely perceived as a conscious sensation, at least under physiological conditions.<sup>10,11</sup> In addition, certain extrinsic sensory neurones subserve a local defensive function in the mucosa inasmuch as they can signal for vascular, secretory and motor reactions if mucosal integrity is challenged by chemical noxae.<sup>12</sup>

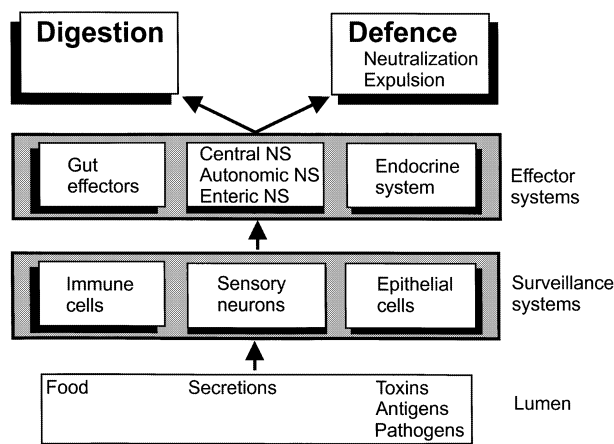
Consistent with the overall homeostatic role of afferent neurones in the gut is their ability to adapt to changes in the GI environment. It seems, however, that sensory neurones also can fall victim to their adaptability if alterations in their sensitivity do not revert to normal once the triggering stimulus is over. Thus, patients with functional bowel disorders (FBDs) such as functional dyspepsia or irritable bowel

syndrome (IBS) suffer from GI pain and discomfort, and there is now ample evidence that hypersensitivity of the extrinsic afferent system is one factor in the complaints of these patients.<sup>13</sup> Although we begin to understand the events whereby GI hyperalgesia may be initiated, we know little about why this hypersensitivity persists and does not abate. We therefore need to study the plasticity of visceral afferent pathways in much more detail before we can fully appreciate the neurological mechanisms of FBDs. It is against this background that the current article focuses on the functional implications of sensory neurones in the alimentary canal and their responses to chemical noxae.

## MULTIPLE POPULATIONS OF SENSORY NEURONES IN THE GUT

Unlike somatic structures and visceral organs, such as the urogenital system, which are supplied by one population of afferent neurones, the alimentary canal is innervated by a multiplicity of sensory neurones that can be classified into four populations according to their origin.<sup>3,4</sup> IPANs that have their cell bodies within the GI tract originate either in the myenteric plexus (Auerbach plexus) or in the submucosal plexus (Meissner plexus) and innervate both the mucosal and muscular layers of the gut.<sup>5,14,15</sup> These two groups of intrinsic sensory neurones are complemented by two groups of extrinsic afferents whose cell bodies lie either in the jugular and nodose ganglia (vagal afferents) or in the dorsal root ganglia (spinal afferents). Importantly, 80–90% of the axons in the vagus nerves are afferent nerve fibres that project to the nucleus tractus solitarius and area postrema of the brainstem, while only a 10–15% minority of the somata in the dorsal root ganglia supplies visceral tissues.<sup>6,8</sup>

The spinal afferent nerve fibres are organized in a segmental manner but, unlike those of somatic afferents, distributed over several spinal segments.<sup>6,8,16</sup> This diffuse termination pattern in the spinal cord explains the diffuse localization 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.<sup>6,8</sup> On their route to the GI tract, spinal afferents pass through the prevertebral ganglia where they give off collaterals to form axodendritic and axosomatic synapses with the sympathetic ganglion cells.<sup>17</sup> Except for particular spatial arrangements in the myenteric plexus and muscle,<sup>9,18</sup> the visceral endings of the vagal and spinal afferents have no end organs or



**Figure 1** Conflicting tasks of the gastrointestinal tract in digesting food and in defending its mucosa against toxins, antigens and pathogens. Surveillance systems, among which are sensory neurones, monitor the luminal contents and activate the appropriate effector systems to proceed with digestion or to neutralize and expel hazardous materials. NS, nervous system.

morphological specializations. Associated mostly with nonmyelinated and some thinly myelinated axons, the extrinsic sensory nerve fibres supply mucosa, submucosa (particularly arterioles), muscle, myenteric plexus and serosa.<sup>3,4,6-9,16</sup> With these projections and their sensory modalities, they can respond to changes of 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.<sup>6-8,16</sup>

### COMMUNICATION FROM INTRINSIC TO EXTRINSIC AFFERENTS

Although the intrinsic and extrinsic afferent neurones of the GI tract are distinct in terms of origin and functional implications, they share a number of characteristics. Thus, both groups of sensory neurones have similar innervation territories in mucosa and muscle, are responsive to both chemical and mechanical stimuli, and share neurochemical traits such as substance P (SP)<sup>3-5</sup> and the vesicular glutamate transporter of type 2.<sup>19</sup> In contrast, only extrinsic afferents seem to be susceptible to the pharmacological actions of capsaicin<sup>12,20</sup> which, for this reason, has been a very useful tool to differentiate between the functional implications of extrinsic and intrinsic sensory neurones in the gut. The many similarities between extrinsic and intrinsic afferents raise questions about the biological significance of two parallel and overlapping sensory systems and any possible interaction between the two systems. While there is evidence that extrinsic afferents communicate to enteric neurones, there is still limited information to infer that intrinsic sensory neurones issue outputs to extrinsic afferents.

The best example of a close association between enteric neurones and extrinsic afferents is that of intraganglionic laminar endings (IGLEs), which relate to dense networks of terminal collaterals of vagal afferents around myenteric ganglia.<sup>9,18</sup> As vagal afferent nerve fibres do not seem to communicate to enteric neurones<sup>21</sup> or pick up information directly from enteric neurones, it is thought that IGLEs represent vagal tension receptors, an inference that is supported by functional investigations.<sup>9,18,22</sup> There is, however, pharmacological evidence that enteric neurones could pass on information to certain vagal afferents via nicotinic acetylcholine (ACh) receptors. Thus, ACh and the nicotinic receptor agonist dimethylphenylpiperazine can stimulate vagal afferent nerve fibres in the rat jejunum<sup>23</sup> much as nicotine can activate vagal afferents from the portal vein.<sup>24</sup> As ACh is the major excitatory transmitter of the ENS, it seems conceivable

that vagal afferent nerve fibres associated with enteric ganglia could directly respond to cholinergic ENS activity. Transmitters such as glutamate or adenosine triphosphate (ATP) might also communicate between neurones of the ENS and extrinsic afferents. It remains to be explored, though, whether information gathered and reinforced by the network of IPANs<sup>5</sup> is in fact communicated to the extrinsic sensory innervation. Despite these possible interactions it is obvious that intrinsic and extrinsic sensory neurones subserve two different roles in GI function. IPANs are essential for the control of digestion by the ENS whereas extrinsic afferents are relevant to body energy, fluid and electrolyte homeostasis but also to the sensation of pain.

### COMMUNICATION FROM EXTRINSIC AFFERENTS TO ENTERIC NEURONES

There is multiple evidence that spinal afferents feed sensory information into the ENS. When stimulated, spinal afferent nerve fibres in the gut release transmitters such as calcitonin gene-related peptide (CGRP), the tachykinins SP and neurokinin A (NKA), nitric oxide (NO) and ATP, which act on enteric neurones and other GI effector systems.<sup>12,25-32</sup> Through these messengers, spinal afferent neurones can bring about motor stimulation or inhibition, electrolyte, mucus and fluid secretion, arteriolar dilatation, increase in venular permeability, degranulation of mast cells and activation of immune cells.<sup>12,26-28,30-32</sup> The vascular and immune effects are summarized under the term neurogenic inflammation.<sup>33</sup> This efferent-like flow of information from extrinsic afferent to enteric neurones and GI effectors is not only a pharmacological oddity but a functionally relevant factor in the activation of the ENS in response to acid challenge of the gastric mucosa,<sup>12,34</sup> exposure of the intestinal mucosa to *Clostridium difficile* toxin A<sup>29,35</sup> or distension.<sup>32</sup> From these implications it is evident that the efferent-like function of sensory neurones comes into play when the integrity of the GI mucosa is threatened.<sup>12</sup>

### RELEVANCE OF EFFERENT-LIKE FUNCTIONS OF SENSORY NEURONES TO GASTROINTESTINAL MUCOSAL INTEGRITY

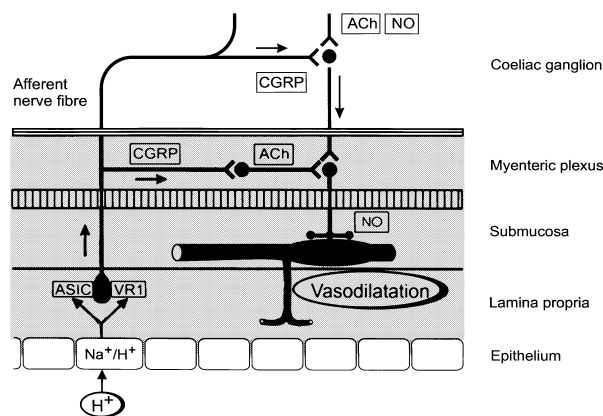
#### Sensory neurones monitor noxae in the gastrointestinal mucosa

Experimental studies in the rat have shown that endogenous or exogenous chemicals threatening GI

mucosal integrity lead to activation of capsaicin-sensitive afferent neurones that through their efferent-like function signal for local protective measures in the mucosa or through their afferent function stimulate autonomic and neuroendocrine mechanisms of homeostasis.<sup>12,36</sup> Hydrochloric acid (HCl) and pepsin are aggressive secretions of the stomach that attack the mucosal tissue if they can overwhelm the mucosal barrier. This is thought to take place when mucosal continuity is disrupted by mechanical forces of digestion, alcohol, nonsteroidal anti-inflammatory drugs or reflux of bile. The surge of acid intruding the lamina propria stimulates spinal afferent nerve fibres<sup>37,38</sup> that via a peripheral mechanism of action increase blood flow through the gastroduodenal mucosa (Fig. 2) and initiate other mechanisms of defence such as an increase in the mucus gel thickness.<sup>39,40</sup>

### Pathways underlying the protective sensory neurone responses to acid challenge

The acid-evoked rise of duodenal mucosal blood flow takes place in parallel with a fall of intracellular pH in the epithelium. It has therefore been suggested that following luminal acid challenge, epithelial cells



**Figure 2** Hypothetical neural pathways whereby influx of acid into the rat gastroduodenal mucosa leads to local vasodilatation. Intracellular acidification of epithelial cells activates a basolateral  $\text{Na}^+/\text{H}^+$  exchange transporter whereby protons are delivered into the lamina propria. Interstitial acidification excites spinal afferent nerve fibres through stimulation of vanilloid receptors of type 1 (VR1) and/or acid-sensing ion channels (ASIC). By releasing calcitonin gene-related peptide (CGRP), these afferent nerve fibres activate a peripheral vasodilator circuit that involves the coeliac ganglion and the myenteric plexus. Besides CGRP, acetylcholine (ACh) acting via nicotinic receptors and nitric oxide (NO) also play an important mediator role.

themselves generate a signal for mucosal vasodilatation, which may be expulsion of  $\text{H}^+$  ions through an amiloride-sensitive  $\text{Na}^+/\text{H}^+$  exchange transporter at the basolateral membrane (Fig. 2).<sup>41</sup> Interstitial acidification, in turn, appears to stimulate acid-sensitive spinal afferents through activation of vanilloid receptors of type 1 (VR1),<sup>42</sup> which are expressed by a large number of dorsal root ganglion cells.<sup>43–45</sup> However, the presence of VR1 on gastric epithelial cells<sup>46</sup> and IPANs<sup>47</sup> raises the possibility that acid stimulates VR1 on mucosal cells and IPANs that in turn signal to CGRP-releasing extrinsic sensory neurones. In addition, bradykinin may be formed in the acid-threatened gastroduodenal mucosa and contribute to sensory neurone stimulation<sup>48,49</sup> whereas the implication of prostaglandins (PGs) is less clear. Thus, PGs do not contribute to the acid-evoked mucosal hyperaemia<sup>42,50</sup> but play a role in the final pathway that increases mucus gel thickness in the acid-challenged mucosa.<sup>40</sup> However, PGs may come into play in the rat gastric mucosa exposed to hypertonic saline. This stimulus releases mucosal PGs, of which only  $\text{PGI}_2$  is able to stimulate CGRP-releasing afferent nerve fibres and thereby to bring about adaptive cytoprotection from ethanol injury.<sup>51</sup>

As acute acid challenge of the gastric mucosa is not signalled to the spinal cord<sup>52</sup> it is thought that the acid-induced rise of mucosal blood flow is mediated by a peripheral vasodilator circuit that involves spinal afferent nerve fibres,<sup>37,38</sup> neuronal structures passing through the coeliac ganglion<sup>53</sup> and, most likely, enteric neurones (Fig. 2).<sup>34</sup> Besides CGRP, which is most likely released from spinal afferents, ACh acting via nicotinic receptors and NO also play important mediator roles.<sup>42,50,54–56</sup> The source of ACh, which may be derived from extrinsic autonomic and/or intrinsic enteric neurones, has not yet been identified. The same applies to NO, which may be formed in neurones and/or endothelial cells. It thus remains to be determined whether nitrergic neurones of the myenteric plexus, which are stimulated by mucosal acid challenge,<sup>34</sup> participate in the functional responses to this stimulus (Fig. 2). Such an implication is not unlikely, given that myenteric neurones of the rat colon are activated by exposure to formalin, acetic acid and other inflammatory stimuli.<sup>57</sup>

### Sensory neurones as local emergency system in the gastrointestinal mucosa

In a pathophysiological perspective, spinal afferents represent an emergency system that by increasing blood flow, thickening the surface mucus gel and enhancing bicarbonate secretion defends the

gastroduodenal mucosa against the onslaught of luminal acid.<sup>12,58</sup> However, this protective system is alarmed under many other harmful conditions, because GI damage due to a variety of injurious factors is attenuated by concomitant stimulation of afferent neurones with capsaicin and exacerbated if these neurones have been defunctionalized.<sup>12,58,59</sup> The same alarm system operates in the human gastroduodenal mucosa<sup>60</sup> and throughout the oesophagus, stomach, small intestine and colon of experimental animals.<sup>12</sup> This defensive role of sensory neurones has important implications for the understanding and therapy of GI mucosal lesions. Firstly, there is evidence that sensory nerve-mediated protection of the GI mucosa is impaired under several pathological conditions including diabetes.<sup>12,58</sup> Secondly, any therapeutic intervention should be designed such that the neural alarm system is maintained or facilitated. The feasibility of this concept is corroborated by the observation that the mucosaprotective effect of several endogenous factors (cholecystokinin, gastrin, epidermal growth factor, PGI<sub>2</sub>) and anti-ulcer drugs (ecabet, lafutidine) involves capsaicin-sensitive afferent neurones.<sup>12,51,58,61,62</sup>

#### RELATIONSHIP BETWEEN THE EFFERENT-LIKE AND AFFERENT FUNCTIONS OF SENSORY NEURONES

As extrinsic afferent neurones subserve an efferent-like and afferent function, the question arises as to how these two functions are interrelated with each other. In addressing this issue it needs to be considered that the two functions are served by different populations of afferent neurones.<sup>36</sup> Thus, the local hyperaemic reaction to gastric mucosal acid challenge and the central signalling of this stimulus are brought about by spinal and vagal afferents, respectively.<sup>38,52,53,63</sup> Despite this functional differentiation it is very probable that the efferent-like effects of sensory neurone stimulation to alter GI motor activity, secretory activity, vascular function and immunological processes modify the sensory gain of chemo- and mechanoreceptive afferent neurones. A further aspect of this interaction relates to the possibility that spinal afferents influence the activity of gut effectors via short-loop intestino-intestinal reflexes that are relayed in the coeliac and mesenteric ganglia. These prevertebral ganglia are important for the fine tuning of the sympathetic output to the GI tract, as they receive input from preganglionic sympathetic neurones, intestinofugal enteric neurones and collaterals of spinal afferent neurones.<sup>17,64,65</sup>

#### SENSITIVITY OF GASTROINTESTINAL AFFERENT NEURONES TO ENDOCRINE AND IMMUNE MEDIATORS

##### Mechano- and chemo-sensitive primary afferent neurones

The mechanosensitivity of intrinsic sensory neurones enables the ENS to react to distortion of the mucosal villi and distension of the gut wall by the luminal contents and thus to regulate GI motility, secretory activity and vascular perfusion according to need.<sup>2</sup> Mechanosensitive vagal and spinal afferents contribute to the autonomic regulation of gut function and to the sensation of pain. Importantly, most mechanosensitive extrinsic afferents of the GI tract are low-threshold sensors that, however, encode distension and other mechanical stimuli over a wide range of innocuous and noxious intensities, while others are high-threshold sensors, as is typical of nociceptors.<sup>16,66,67</sup> Whereas subliminal distension of the colorectal region is signalled to the cerebral cortex without evoking any sensation,<sup>11</sup> distension of the stomach, small intestine or colon beyond a certain level is perceived as painful.<sup>6-8</sup> Both low- and high-threshold mechanosensors can sensitize under conditions of inflammation,<sup>16,67</sup> in which case normal distension levels may be encoded at an intensity that causes discomfort or pain. In this article, however, emphasis is laid on the pathophysiological implications of chemosensitive afferents, given that sensory neurones in the gut are continuously exposed to a variety of endogenous and exogenous chemicals.

##### Sensory neurone responses to mediators of enteroendocrine cells

Unlike sensory nerve fibre endings, which are located in the lamina propria behind the GI surface epithelium, enteroendocrine cells are interspersed between the epithelial cells<sup>3,4</sup> and thus are strategically positioned to survey the GI lumen. Functioning like taste buds, these cells serve as interface between the GI lumen and nerve fibres in the lamina propria<sup>3,68</sup> and thereby enable afferent neurones to detect luminal stimuli that otherwise could not be encoded by their axons. The humoral mediators whereby enteroendocrine cells communicate with sensory nerve fibres comprise 5-hydroxytryptamine (5-HT), cholecystokinin, secretin, somatostatin and corticotropin-releasing factor. The enterochromaffin cells are the major source of 5-HT in the body and release this amine in response to various luminal stimuli including hyperosmolarity,

carbohydrates, mechanical distortion of the mucosal villi, bacterial products such as cholera toxin, cytostatic drugs such as cisplatin and mucosal injury.<sup>4,69-75</sup> 5-HT, in turn, can activate IPANs and other types of enteric neurones as well as vagal and spinal afferents, which express various subtypes of 5-HT receptors, those on extrinsic afferents being preferentially of the 5-HT<sub>3</sub> type.<sup>4,69,73-78</sup>

Cholecystokinin is released from particular enteroendocrine cells in the duodenum in response to products of fat and protein breakdown.<sup>68,79</sup> The peptide has been found to stimulate vagal afferents involved in satiation, reflex inhibition of gastric motility and emptying, reflex increase in gastric blood flow and mucosal protection.<sup>76,80-84</sup> These effects are mediated by CCK<sub>1</sub> (CCK<sub>A</sub>) receptors,<sup>76,83,84</sup> which is consistent with the expression of this receptor type on vagal afferents of the rat.<sup>85,86</sup> Secretin, being released by gastric acid from endocrine S cells in the proximal small intestine, leads to stimulation of vagal afferents and subsequent inhibition of gastric contractility and emptying via a vagovagal reflex.<sup>87,88</sup> The action of somatostatin, which in the gut is expressed by endocrine D cells and enteric neurones, is particularly noteworthy because this peptide inhibits the activity of chemo- and mechanosensitive spinal afferents in the rat jejunum via activation of somatostatin sst<sub>2</sub> receptors.<sup>89</sup> Corticotropin-releasing factor may be released from enterochromaffin cells or immune cells of the GI mucosa and could increase extrinsic afferent nerve activity as suggested by a study in human volunteers.<sup>90</sup>

### Sensory neurone responses to immune mediators

The GI tract harbours not only the largest number of neurones outside the central nervous system (CNS) but, in anticipation of the continuous threats from the lumen, also contains the largest collection of immune cells in the body.<sup>3,4,91</sup> The gut-associated lymphoid tissue comprises M cells, which are modified enterocytes that sample antigens from the lumen and may even be involved in the transepithelial transport of infectious prions,<sup>92</sup> lymphocytes that may either occur in aggregates (such as in Peyer's patches) or lie loosely scattered in the epithelium and lamina propria, and immune-associated cells including macrophages, eosinophils, neutrophil granulocytes and mast cells.<sup>3,4,91,93</sup> In addition, many epithelial cells synthesize and secrete chemokines (e.g. interleukin-8, macrophage inflammatory protein-2) in response to an attack by microorganisms.<sup>94-96</sup> Whenever the GI mucosa is affected by microbial infection, allergy, inflammation or other types of injury, immune cells are activated and

release a host of pro- and anti-inflammatory mediators such as cytokines, PGs, leukotrienes, proteases, bradykinin, histamine and 5-HT.<sup>3,4,71,97</sup> Most of these factors have the ability to excite sensory neurones, in the short term, or to alter their sensitivity, in the long term. While the acute messenger links between immune cells and sensory neurones are increasingly disclosed, little is still known about their long-term interactions, which are more relevant to understanding immune system-sensory neurone communication in the diseased gut.

Intestinal anaphylaxis (type I hypersensitivity) leads to degranulation of mast cells that release a cocktail of mediators including histamine, serine proteases and PGs. All these messengers are neuroactive, and histamine excites both vagal and spinal afferents via activation of histamine H<sub>1</sub> receptors.<sup>71,97</sup> Serine proteases such as mast cell tryptase, trypsin and thrombin stimulate a particular group of cell surface receptors termed protease-activated receptors (PARs).<sup>98</sup> Of the four PARs identified thus far, PAR-1 and PAR-2 are expressed by spinal afferents containing CGRP, which is consistent with the ability of a PAR-2 agonist to excite afferent fibres in jejunal mesenteric nerves, afferent nerve fibres in the pancreatic duct and dorsal root ganglion neurones in culture.<sup>99-101</sup> Moreover, stimulation of PAR-2 on sensory neurones in the rat stomach releases CGRP and tachykinins, which enhance mucus secretion and protect the mucosa from injury due to acid, ethanol and indomethacin.<sup>102</sup> Importantly, activation of PARs not only causes acute stimulation of sensory neurones but also gives rise to prolonged hyperalgesia.<sup>103</sup> Thus, stimulation of mucosal PAR-2 in the rat colon brings about a delayed hypersensitivity to colorectal distension and administration of a PAR-2 agonist into the pancreatic duct sensitizes spinal afferents to the excitatory effect of capsaicin.<sup>100,104,105</sup>

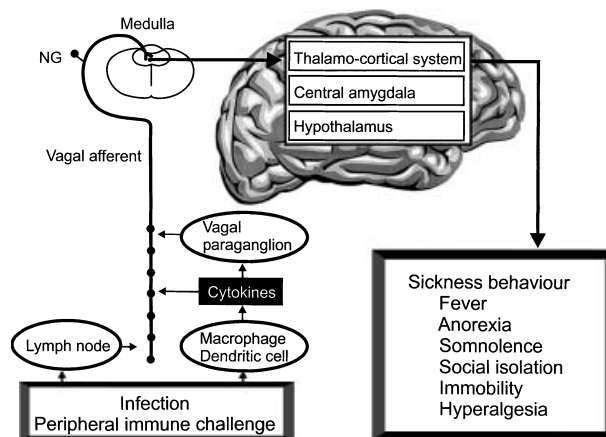
PGs including PGE<sub>2</sub> and PGI<sub>2</sub> are key mediators of hyperalgesia, which is in keeping with the expression of EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3C</sub>, EP<sub>4</sub> and IP receptors on primary sensory neurones.<sup>106,107</sup> Produced by a variety of cells, PGs such as PGE<sub>2</sub> can excite mesenteric afferent nerve fibres from the rat jejunum.<sup>108</sup> The initial phase of this stimulant action is mediated by axonal EP<sub>1</sub> receptors, whereas the delayed phase seems to be due to EP<sub>2</sub> receptor-mediated sensitization of nerve fibres.<sup>108</sup> PGs also sensitize visceral afferents to other algescic substances such as bradykinin that *per se* increases the discharge of serosal afferents from the rat jejunum by activation of bradykinin B<sub>2</sub> receptors.<sup>109</sup> While in cultured dorsal root ganglion neurones both EP<sub>3C</sub> and EP<sub>4</sub> receptors contribute to the PGE<sub>2</sub>-induced

sensitization,<sup>107</sup> PGE<sub>2</sub>-induced sensitization of testispermatic afferent nerve fibres to bradykinin *in vivo* seems to be mediated by EP<sub>3</sub> receptors while that to heat involves EP<sub>2</sub> receptors.<sup>110</sup> In contrast, both IP and EP<sub>3</sub> receptors participate in the endotoxin-evoked sensitization of peritoneal afferents in mice as assessed by the writhing response to intraperitoneal acetic acid.<sup>111</sup>

The expression of proinflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) in monocytes and macrophages is elevated in inflammatory bowel disease, in which their levels reflect the severity of disease.<sup>112</sup> In addition to macrophages,<sup>113</sup> enteroglia cells<sup>114</sup> and intestinal smooth muscle cells<sup>115</sup> are further sources of cytokines that target intrinsic as well as extrinsic neurones. Given that IL-1 $\beta$  and IL-6 are formed not only after immunological challenge but also after ischaemia,<sup>116</sup> acid-induced gastric mucosal injury<sup>117,118</sup> and surgical trauma,<sup>119</sup> it would seem that cytokines come into play in a wide range of conditions. Apart from influencing the activity of enteric and sympathetic neurones,<sup>114</sup> cytokines lead to excitation and/or sensitization of extrinsic afferents in the GI tract. Thus, IL-1 $\beta$  sensitizes splanchnic afferents to the excitatory effects of mesenteric ischaemia and histamine and, at high dosage, directly excites them via stimulation of IL-1 type I receptors.<sup>116</sup> Cytokines may also be responsible for the acute sensitization of jejunal afferent neurones to mechanical and chemical stimuli, which in the rat is caused by *Escherichia coli* endotoxin.<sup>120</sup>

### ROLE OF VAGAL AFFERENTS IN THE COMMUNICATION BETWEEN IMMUNE SYSTEM AND BRAIN

Due to their sensitivity to pro-inflammatory cytokines, vagal afferents take a particular position in the communication between the peripheral immune system and the CNS (Fig. 3). Thus, systemic bacterial lipopolysaccharide (endotoxin) is primarily transported to the liver where it induces the release of IL-1 $\beta$  from Kupffer cells (macrophage-like cells to screen blood and lymph) and thereby causes stimulation of afferents in the hepatic branch of the vagus nerve.<sup>121,122</sup> Moreover, the abdominal vagus is associated with paraganglia and connective tissue containing macrophages and dendritic cells that respond to intraperitoneal administration of endotoxin with synthesis of IL-1 $\beta$  (Fig. 3).<sup>123,124</sup> In addition, the abdominal paraganglia of the vagus nerve contain glomus-like cells that have IL-1 receptors, are innervated by vagal afferents and



**Figure 3** Role of vagal afferents in the communication between peripheral immune system and brain and in the sickness behaviour evoked by infection. NG, nodose ganglion.

may hence serve as chemosensory accessory cells of vagal afferents.<sup>124,125</sup> Vagal afferents also supply abdominal lymph nodes, which represent another interface with the visceral immune system.<sup>124</sup>

IL-1 $\beta$  leads to increased firing in vagal afferents and enhances the expression of c-fos, a marker of neuronal excitation, in the nodose ganglion and in the nucleus tractus solitarius, the central termination area of vagal sensory neurones.<sup>106,124,126–128</sup> As IL-1 receptors of type I are expressed by nodose ganglion cells, it seems as if IL-1 $\beta$  can excite vagal afferents by a direct action on the axons (Fig. 3) although PGs acting via EP<sub>3</sub> receptors and cholecystokinin acting via CCK<sub>1</sub> receptors may also contribute.<sup>106,127,129</sup> Being responsive to peripheral IL-1 $\beta$ , vagal afferents participate in the behavioural responses to infection and inflammation, which comprise fever, anorexia, somnolence, decrease in locomotor activity, decrease in social exploration and hyperalgesia (Fig. 3).<sup>124,130</sup> Accordingly, certain features of this 'sickness behaviour' or cluster of 'illness responses' are attenuated by subdiaphragmatic vagotomy, although circulating proinflammatory cytokines can access the brain also via circumventricular organs that are devoid of a blood-brain barrier.<sup>124,130</sup>

### ROLE OF VAGAL AFFERENTS IN ABDOMINAL PAIN

The involvement of vagal afferents in the cytokine-induced sickness behaviour (Fig. 3)<sup>124,130</sup> is of obvious relevance to understanding visceral sensation in health and disease. After it has long been agreed that vagal sensory neurones do not play any role in abdominal pain, there is now growing awareness that

these neurones can make a distinct contribution to disease-related alterations in visceral sensation.<sup>16,131</sup> The participation of vagal afferents in nausea and emesis and in cytokine-evoked illness responses<sup>69,124,130</sup> corroborates the view that sensory neurones in the vagus nerve may determine the autonomic, endocrine, emotional-affective and behavioural aspects of abdominal nociception (Fig. 4).<sup>132</sup> Strong electrical stimulation of the vagus nerve for the treatment of medically intractable epilepsy can even give rise to the sensation of overt pain.<sup>133</sup> It is in keeping with a role in pain-related communication that most (80–90%) vagal axons are afferent nerve fibres,<sup>6,8</sup> making the vagus nerve the largest visceral sensory nerve in the body.<sup>134</sup> The continuous flow of information, which the vagus nerve conveys from the gut to the brain, is thought to be relevant not only to the autonomic regulation of GI function but also to the interpretation of external sensory inputs, attitude and behaviour (Fig. 4).<sup>134</sup> Thus, the afferent part of the vagus nerve has even been suggested to mediate the sixth sense.<sup>134</sup>

A role of vagal afferents in the affective and autonomic, rather than perceptive, aspects of gastric nociception has been borne out by a study of gastric acid noxae. Exposure of the rat gastric mucosa to a minimally injurious concentration of HCl leads to expression of c-Fos in the brainstem, but not spinal cord.<sup>52</sup> The gastric input to the brainstem is passed on to nuclei of the thalamus, hypothalamus (paraventricular and supraoptic nucleus) and limbic system (central amygdala and habenula).<sup>63</sup> As there is no activation of

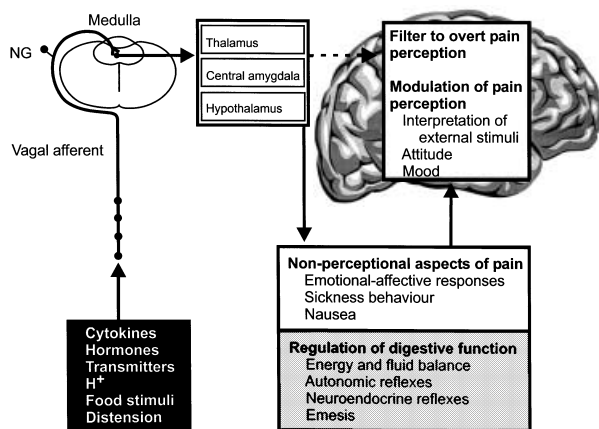
the insular cortex, the major cerebral representation area of afferent input from the stomach, it would appear that vagal afferent signalling of acute gastric acid noxae does not evoke perception of pain but leads to activation of subcortical brain nuclei involved in autonomic, endocrine, emotional and behavioural reactions (Fig. 4).<sup>63</sup> In a wider perspective it can thus be hypothesized that vagal and spinal afferents mediate different homeostatic reactions to a dangerous acid onslaught in the rat stomach and ferret oesophagus inasmuch as capsaicin-sensitive spinal afferents alarm local defence mechanisms whereas capsaicin-insensitive vagal afferents signal to the CNS.<sup>12,36,52,135–137</sup> This functional dissociation is in keeping with the notion that most vagal afferents including those responding to IL-1 $\beta$  and trauma are resistant to the pharmacological action of capsaicin.<sup>52,136–141</sup>

## VISCERAL HYPERALGESIA IN FUNCTIONAL BOWEL DISORDERS

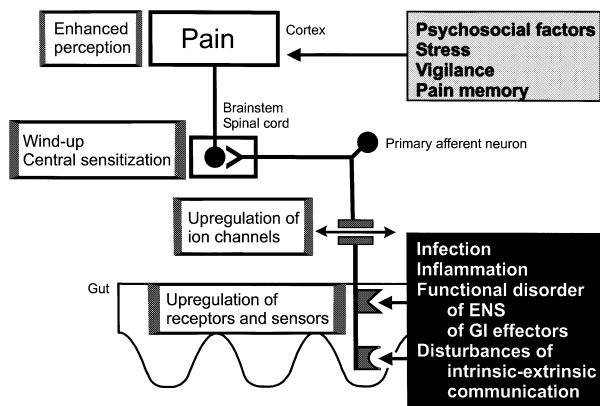
### Mechanical and chemical hypersensitivity in functional dyspepsia and irritable bowel syndrome

Although there is a continuous flow of information from the gut to the brain, this input is normally processed only in autonomic and neuroendocrine circuits and does not reach the level of consciousness.<sup>10,11,134</sup> Abdominal pain is thus a sensation whose physiological or pathological meaning is not immediately clear. While, for instance, epigastric symptoms may be a warning sign<sup>142</sup> to abstain from further food intake or to avoid certain types of food, in most cases of GI pain we do not have a behavioural repertoire with which to appropriately react to abnormal sensations from the gut. FBDs such as functional dyspepsia and IBS are currently defined by their chronic or recurrent abdominal symptom patterns without an organic cause identifiable by conventional diagnostic means.<sup>13</sup> The functional abnormalities comprise alterations in motor and secretory activity, dysreflexia and hyperalgesia, but the underlying causes and mechanisms are largely unknown and the therapeutic options limited.<sup>13,142–144</sup>

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 a distorted fashion because the sensory gain of afferent neurones or the central gain of afferent input from the GI tract is set abnormally high (Fig. 5). Evidence for an association of FBDs with GI hyperalgesia has come from clinical observations that dyspeptic patients are hyper-



**Figure 4** Role of vagal afferents in abdominal nociception. Vagal afferent pathways signal innocuous and noxious stimuli from the gut to subcortical nuclei and thereby contribute to various nonperceptual aspects of abdominal nociception. Through these effects, vagal afferent pathways modulate the perception of pain although they do not themselves give rise to the perception of pain. NG, nodose ganglion.



**Figure 5** Mechanisms underlying visceral hyperalgesia in functional bowel disorders. ENS, enteric nervous system; GI, gastrointestinal.

sensitive to gastric distension as are IBS patients to colorectal distension.<sup>10,13,142–146</sup> Patients with functional dyspepsia are also liable to experience discomfort and pain in response to chemical stimuli such as fat and acid.<sup>147–149</sup> Although gastric acid secretion is in the normal range,<sup>150</sup> administration of exogenous acid to the stomach or duodenum of dyspeptic patients can evoke exaggerated sensations<sup>148,149</sup> and lower the threshold of mechanosensitive afferents.<sup>151</sup> These findings and the beneficial effect of antisecretory treatment in some patients suggest that acid hypersensitivity, but not acid hypersecretion, is a factor in functional dyspepsia.<sup>144,152–154</sup> In this context it is important to note that acid also contributes to gastric and duodenal ulcer pain<sup>155,156</sup> and that gastro-oesophageal reflux disease is associated with enhanced oesophageal sensitivity to acid, but not distension.<sup>157</sup>

### Inflammation as a risk factor in gastrointestinal hypersensitivity

As infectious gastroenteritis has been recognized as a risk factor for IBS,<sup>114,158–160</sup> it is conceivable that inflammatory processes can initiate long-lasting changes in bowel function and nociceptive afferent pathways (Fig. 5), alterations that are ultimately responsible for the hyperalgesia that is characteristic of many FBD patients. By the time postdysenteric IBS is diagnosed, no overt inflammation is seen in the colon, although the number of lymphocytes, mast cells and 5-HT-containing enteroendocrine cells is elevated in intestinal biopsies as is the permeability of the mucosa.<sup>158,160,161</sup> The hypothesis that enteritis gives rise to GI hyperalgesia is supported by experimental observations in the rat and mouse in which gastric or colonic inflamma-

tion causes hypersensitivity to distension.<sup>71,162–164</sup> Experimentally induced states of GI hyperalgesia can become permanent if the priming insult (e.g. irritation of the colon, maternal separation) is experienced early in life.<sup>165,166</sup> A definite role of *Helicobacter pylori* infection in functional dyspepsia has not yet been ascertained,<sup>142,144</sup> although it needs to be noted that dyspeptic patients with a very intense immune reaction to *Helicobacter pylori* infection as deduced from a high antibody titre have significantly lowered sensory thresholds to duodenal distension.<sup>167</sup>

If infection and inflammation are risk factors for FBDs, it is obvious to hypothesize that pro-inflammatory cytokines and mediators are relevant to the development of persistent hyperalgesia. This hypothesis is supported by experimental findings that, for example, the excitatory effect of PGE<sub>2</sub> on mesenteric afferent nerve fibres supplying the rat jejunum is enhanced for at least 20 h following systemic administration of IL-1 $\beta$ .<sup>129</sup> Hypersensitivity is not confined to spinal afferents but concerns vagal afferents also.<sup>124,168</sup> Thus, systemic administration of IL-1 $\beta$  and TNF- $\alpha$  leads to prolonged sensitization of vagal afferent pathways to gastric acid challenge, a change that is seen as long as 4 days post-treatment.<sup>128</sup> An important issue that has remained unsolved relates to the question why some patients affected with infectious gastroenteritis develop FBDs whereas others do not. One clue to this problem may come from the observation that some patients with IBS have a genetic background that is associated with a diminished production of anti-inflammatory cytokines such as IL-10.<sup>169</sup>

### Long-term sensitization of primary afferent neurones

The available evidence indicates that the GI hypersensitivity associated with FBDs involves sensitization of primary afferent neurones (peripheral hyperalgesia) and nociceptive pathways within the CNS (central hyperalgesia).<sup>10,16,71,170,171</sup> This concept identifies extrinsic afferent neurones supplying the gut as a prime target at which novel therapies may be aimed.<sup>97,172</sup> The question therefore arises as to the mechanisms whereby nociceptive afferents are sensitized and the state of hypersensitivity is maintained and to the therapeutic possibilities whereby sensitization can be prevented or reversed. Activation of nociceptive afferents is followed by activity-dependent plasticity, which means that repeated exposure of sensory nerve terminals to noxious stimuli increases their response magnitude and/or decreases their response threshold.<sup>16,173</sup> As long

as it is reversible, sensitization of nociceptors results from modulation of nerve fibre excitability via post-translational changes such as phosphorylation of receptors, ion channels or associated regulatory proteins.<sup>173</sup> 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 as well as changes in the phenotype, structure, connectivity and survival of afferent neurones (Fig. 5).<sup>173</sup> There is indirect evidence that sensory neurone transmitters are upregulated in painful functional dyspepsia as deduced from a rise of the SP and CGRP concentrations in the gastric mucosa of patients with this disorder.<sup>174,175</sup> Experimental studies suggest that neurotrophins and cytokines, which are generated in the inflamed gut, are important factors for the long-term sensitization of primary afferent neurones.<sup>124,128,129,173,176,177</sup>

As the gut is increasingly considered as a neurological organ,<sup>2</sup> it is likely that FBDs, which are still defined by their abdominal symptom patterns, will eventually be regarded as neurological bowel disorders<sup>74,178</sup> that are defined in terms of functional disturbances of ENS, GI effectors, extrinsic autonomic neurones, extrinsic afferent neurones and central pain processing systems as well as perturbed communication between these systems (Fig. 5). Experimental and clinical evidence demonstrates that psychosocial factors, vigilance, pain memory, stress and other life events also play a role in the symptomatology of FBDs.<sup>13,165,166,179</sup> Information flow along the brain-gut-brain axis not only explains why factors impacting primarily on the brain can have a profound influence on GI function<sup>13,179</sup> but also illustrates that exaggerated signalling from the gut to the brain may appreciably modify the emotional-affective status of FBD patients.

## CAPSAICIN RECEPTORS AS DRUG TARGETS IN VISCERAL HYPERALGESIA

### Vanilloid receptor ion channels on primary afferent neurones in the gut

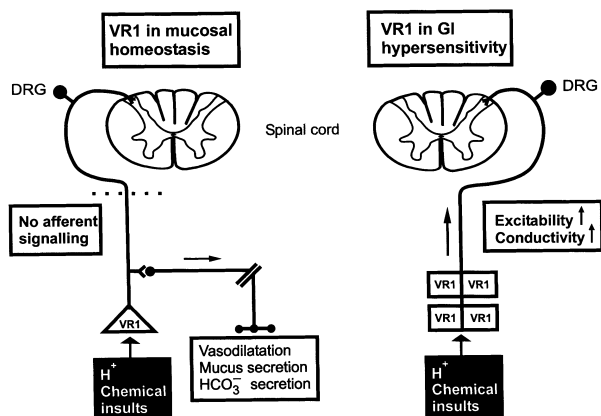
Owing to its apparently selective action on primary afferent neurones, the vanilloid capsaicin has long been used as a probe to examine extrinsic afferent neurone functions in the gut.<sup>12,20,28,180,181</sup> We now know that the cell-specific effects of capsaicin are mediated by VR1, which is a nonselective cation channel with high permeability for Ca<sup>2+</sup>. Consisting of six transmembrane domains,<sup>43</sup> VR1 forms multimers such that the native capsaicin receptor is a VR1 homotetramer,

which may contribute to the complexity of VR1 pharmacology.<sup>182</sup> VR1 operates as a sensor of potentially harmful stimuli including noxious heat, the vanilloid capsaicin, H<sup>+</sup> ions, arachidonyl ethanolamide (anandamide), 12-(S)-hydroperoxyeicosatetraenoic acid and related lipoxygenase products.<sup>43,181–184</sup> Vanilloids, anandamide and lipoxygenase products activate VR1 by interacting with an intracellular site of the cation channel, whereas H<sup>+</sup> targets an extracellular domain.<sup>184–187</sup> Importantly, VR1 is not only a polymodal detector of chemical noxae but, conceivably, also an important factor in the sensitization of afferent neurones, given that many algescic substances including PGE<sub>2</sub>, bradykinin and ATP can enhance the probability of agonist-induced VR1 channel gating through phosphorylation of VR1 or alleviating repression of VR1 activity.<sup>188–190</sup>

Although extensive pharmacological studies indicate that VR1 is almost exclusively associated with primary afferent neurones,<sup>20,180,181</sup> this ion channel is also present in the CNS,<sup>191</sup> albeit at a comparatively low level<sup>192</sup> and with properties somewhat different from VR1 on dorsal root ganglion neurones.<sup>193</sup> Most spinal afferents supplying the rodent gut are sensitive to the sensory neurone-blocking action of capsaicin,<sup>12</sup> whereas the proportion of capsaicin-sensitive vagal afferents projecting to the oesophagus and stomach is approximately 30%.<sup>138,139</sup> From pharmacological studies in the gut it has been deduced that VR1 is confined to extrinsic afferent neurones but most probably absent from enteric neurones.<sup>20,28,43–45</sup> However, VR1-like immunoreactivity has been localized not only to extrinsic nerve fibres but also to enteric neurones, particularly IPANs, interstitial cells of Cajal and epithelial cells of the rat GI tract,<sup>46,47,194,195</sup> whereas in the human colon, VR1 appears to be confined to seemingly extrinsic nerve fibres.<sup>196</sup> The functional implications of VR1 expressed by IPANs and non-neural cells of the gut await to be elucidated.

### Antinociceptive action of capsaicin receptor antagonists at the cost of mucosal vulnerability?

There is ample evidence that capsaicin-induced activation of VR1 stimulates extrinsic afferents of the gut<sup>12,109,139,197</sup> and, at excessive concentrations, evokes abdominal pain in animals and humans.<sup>198–202</sup> These findings, the molecular properties of VR1 as a polymodal detector of chemical noxae<sup>43,181–184</sup> and the facilitation of VR1 gating by inflammatory mediators,<sup>188–190</sup> point to VR1 as a pharmacologically relevant target in abdominal pain. Such a role may be of particular significance under conditions of GI



**Figure 6** Hypothetical diversity in the functions of vanilloid receptors of type 1 (VR1) in the maintenance of gastrointestinal (GI) mucosal integrity and GI hyperalgesia. Being a polymodal detector of injurious chemicals, VR1 on sensory neurones with an efferent-like function is involved in the alarm of local protective reactions in the GI mucosa. The abdominal hyperalgesia associated with functional bowel disorders may in part be due to upregulation and functional modification of VR1 on spinal afferents that signal to the central nervous system. DRG, dorsal root ganglion.

inflammation, given that VR1 activity is enhanced by neurotrophic factors<sup>190,203,204</sup> whose production is boosted in inflamed tissues. Indeed, the expression of VR1-like immunoreactivity on nerve fibres in the colon is upregulated in patients with inflammatory bowel disease,<sup>205</sup> which is in keeping with an enhanced axonal transport of VR1 into afferent nerve terminals supplying inflamed tissues.<sup>206</sup>

Against this background it can be envisaged that blockade of VR1 by capsaicin receptor antagonists is an efficacious way to reverse GI hyperalgesia. While VR1 antagonists have not yet been employed to treat abdominal pain, such an approach appears worth pursuing because daily intragastric administration of capsaicin (1.75 mg) for 5 weeks has been found to significantly reduce epigastric pain and other symptoms of functional dyspepsia.<sup>207</sup> The beneficial effect of this treatment is likely due to impairment of VR1-expressing nociceptive afferent neurones. As, however, VR1 is involved in the monitoring of acid backdiffusion in the gastroduodenal region<sup>37,42</sup> and capsaicin-sensitive afferent nerve fibres subservise a protective function in the GI mucosa,<sup>12,51,58,61,62</sup> the question arises as to whether GI pain treatment with VR1 antagonists is liable to enhance GI mucosal vulnerability. In addressing this issue, it will be important to find out whether VR1 involved in GI mucosal protection can pharmacologically be differentiated from VR1 mediating GI hyperalgesia (Fig. 6). There is room to

speculate that, in painful FBDs, the number of VR1 on nociceptive afferent nerve fibres is upregulated and its molecular structure altered such that the pharmacological properties of VR1 mediating hyperalgesia differ from VR1 mediating mucosal protection (Fig. 6). Furthermore, mucosal defence and pain signalling may be brought about by different sets of afferent neurones.<sup>36,131,135</sup>

VR1 is just one example of several receptors and ion channels that are selectively expressed by extrinsic afferent neurones and are explored as potential targets for novel GI pain treatments. Being reviewed in detail elsewhere,<sup>97,172</sup> these opportunities comprise, e.g. acid-sensing ion channels, P2X<sub>3</sub> and other purinoceptors, 5-HT<sub>3</sub> receptors and tetrodotoxin-resistant sodium channels (Na<sub>v</sub>1.8). In assessing the therapeutic significance of sensory neurone-specific membrane proteins it will be important to determine whether their number, subunit composition and functional properties are persistently altered in FBDs.<sup>16</sup>

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