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Review

Gastrointestinal afferents as targets of novel drugs for the treatment of functional bowel disorders and visceral pain

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Abstract

An intricate surveillance network consisting of enteroendocrine cells, immune cells and sensory nerve fibres monitors the luminal and interstitial environment in the alimentary canal. Functional bowel disorders are characterized by persistent alterations in digestive regulation and gastrointestinal discomfort and pain. Visceral hyperalgesia may arise from an exaggerated sensitivity of peripheral afferent nerve fibres and/or a distorted processing and representation of gut signals in the brain. Novel strategies to treat these sensory bowel disorders are therefore targeted at primary afferent nerve fibres. These neurons express a number of molecular traits including transmitters, receptors and ion channels that are specific to them and whose number and/or behaviour may be altered in chronic visceral pain. The targets under consideration comprise vanilloid receptor ion channels, acid-sensing ion channels, sensory neuron-specific Na^+ channels, $P2X_3$ purinoceptors, 5-hydroxytryptamine (5-HT), $5\text{-}HT_3$ and $5\text{-}HT_4$ receptors, cholecystokinin CCK_1 receptors, bradykinin and prostaglandin receptors, glutamate receptors, tachykinin and calcitonin gene-related peptide receptors as well as peripheral opioid and cannabinoid receptors. The utility of sensory neuron-targeting drugs in functional bowel disorders will critically depend on the compounds' selectivity of action for afferent versus enteric or central neurons.

Keywords: Sensory neuron; Enteric nervous system; Visceral pain; Visceral hyperalgesia; Functional bowel disorder; Capsaicin receptor; Purinoceptor; Neuropeptide receptor

1. Introduction

Functional bowel disorders such as non-cardiac chest pain, functional (nonulcer) dyspepsia and irritable bowel syndrome are defined by chronic or recurrent abdominal symptom patterns in the absence of an identifiable organic cause. Common to all functional bowel disorders is that the patients complain of sensory discomfort, which covers a wide spectrum of sensations ranging from mild uneasiness to overt pain. Although multiple pathogenic mechanisms underlie the symptoms of functional bowel disorders (Wood et al., 1999; Drossman et al., 2000), it is ultimately sensory neurons that notify the brain of pathological events in the gastrointestinal tract. Alternatively, the afferent nervous system may itself create anomalous sensations if it responds inadequately to physiological processes in the gut, a consideration encompassed in the hypothesis that *visceral hypersensitivity* is in part responsible for many functional bowel disorders (Buéno et al., 1997; De Ponti and Malagelada, 1998; Drossman et al., 2000).

The comprehension of "gut feelings" requires knowledge not only of the afferent innervation of the alimentary canal and their transduction properties but also of the interactions between these sensors and the enteric, central, autonomic, endocrine and immune control systems of digestion. Any change in the gastrointestinal effector systems alters the chemical and mechanical environment of gastrointestinal sensors and hence influences their receptive gain. Given the high incidence of functional bowel disorders, the understanding of visceral sensation and nociception is a particular challenge for pathophysiology, pharmacology and clinical medicine alike. In the face of several reviews on visceral hyperalgesia (Cervero, 1994; Mayer and Gebhart, 1994; Buéno et al., 1997; De Ponti and Malagelada, 1998), this article focuses on important pharmacological properties of gastrointestinal afferent neurons and highlights a number of molecular targets that may be exploited in the therapy of functional bowel disorders and gastrointestinal pain.

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2. Multiple sensory systems in the gut

2.1. Four groups of sensory neurons in the gastrointestinal tract

Unlike other visceral organs, the alimentary canal is innervated by *four* different classes of afferent neurons (Fig. 1). Besides two groups of *intrinsic* sensory neurons which have their cell bodies within the gastrointestinal tract and originate in the myenteric or submucosal plexus, there are two groups of *extrinsic* sensory neurons: vagal and spinal afferents (Furness et al., 1998). While the intrinsic sensory neurons are part of the enteric nervous system (ENS) and supply this brain in the gut with information that is required to regulate digestion according to need, extrinsic afferents supply the central nervous system (CNS) with information that is relevant to body energy, fluid and electrolyte homeostasis, tissue integrity and the sensation of discomfort and pain. The vanilloid capsaicin has been a very useful pharmacological tool to differentiate between intrinsic and extrinsic sensory neurons, because only extrinsic afferents are susceptible to the excitatory and sensory neuron-blocking actions of this compound (Holzer, 1991, 1999).

2.2. Extrinsic sensory innervation of the gut

The vagal and spinal afferent neurons supplying the gut (Fig. 1) differ not only in their origin but also in a number of neurochemical and functional properties (Zhuo et al., 1997; Holzer, 1999). It is important to realize that 75–90% of the axons in the vagus nerves are afferent nerve fibres that originate from the jugular and nodose ganglia and project to the medullary brainstem (Grundy and Scratcherd, 1989; Sengupta and Gebhart, 1994; Berthoud and Neuhuber, 2000). The spinal sensory neurons have their cell bodies in the dorsal root ganglia, but only a 10–15% minority of the somata supplies visceral tissues (Grundy and Scratcherd, 1989; Sengupta and Gebhart, 1994). The central projections of these afferents terminate in distinct laminae of the dorsal spinal cord where they are organized in a segmental manner but, unlike those of somatic afferents, distributed over several spinal segments (Gebhart, 2000). This diffuse termination pattern explains the poor localization of visceral sensations, and the convergence of visceral and somatic afferents in the spinal cord accounts for the referral of visceral pain to somatic structures (Sengupta and Gebhart, 1994).

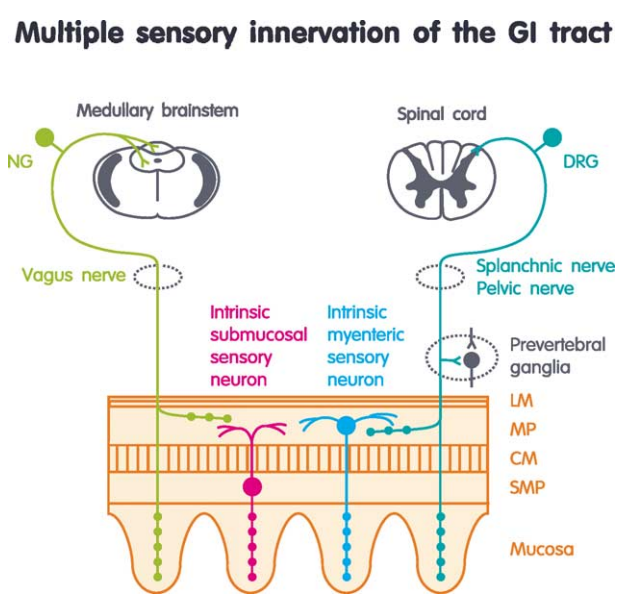

Fig. 1. Sensory innervation of the gastrointestinal (GI) tract by intrinsic and extrinsic afferents. These include two populations of intrinsic sensory neurons, which originate in the submucosal plexus (SMP) and myenteric plexus (MP) of the ENS. The two populations of extrinsic sensory neurons are vagal afferents originating from the nodose ganglia (NG) and spinal afferents originating from the dorsal root ganglia (DRG). CM, circular muscle; LM, longitudinal muscle.

The spinal afferents reach the gastrointestinal tract via the splanchnic and pelvic nerves in which they constitute 10–30% of all nerve fibres (Grundy and Scratcherd, 1989; Sengupta and Gebhart, 1994). On this route, the axons pass through the prevertebral ganglia (Fig. 1) where they give off collaterals to form synapses with the sympathetic ganglion cells (Szurszewski and Miller, 1994). Associated mostly with unmyelinated and some thinly myelinated axons, vagal and spinal afferents supply mucosa, submucosa (particularly arterioles), muscle, myenteric plexus and serosa (Cervero, 1994; Furness et al., 1998; Berthoud and Neuhuber, 2000). With these projections, 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 (Cervero, 1994; Gebhart, 2000).

Apart from signalling to the CNS, certain spinal afferents are specialized in playing an efferent-like function in the gastrointestinal tract. By releasing transmitters such as calcitonin gene-related peptide (CGRP), the tachykinins substance P and neurokinin A, nitric oxide (NO) and/or ATP from their peripheral endings, these afferent neurons influence the activity of the ENS and the gastrointestinal effector systems (Maggi, 1995; Holzer, 1998a,b; Barthó et al., 1999). The sensory neuron-induced functional changes include stimulation or inhibition of motility, secretion of bicarbonate, fluid and mucus, dilatation of arterioles and increase in venular permeability. In so doing, spinal afferents govern protective mechanisms in the gut. If, for instance, the gastroduodenal mucosa is irritated by alcohol or nonsteroidal antiinflammatory drugs, backdiffusion of acid into the lamina propria leads via stimulation of spinal afferents to vasodilatation and activation of other defence mechanisms (Holzer, 1998a). The hyperaemic reaction helps buffering the intruding acid, prevents the formation of ulcers and assists in the healing of mucosal lesions (Holzer et al., 1991; Takeuchi et al., 1994).

2.3. Interactions between intrinsic and extrinsic afferent neurons of the gut

Intrinsic and extrinsic afferent neurons of the gastrointestinal tract share a number of sensory properties and have similar innervation territories in mucosa and muscle. A subgroup of vagal afferents forms dense networks of terminal collaterals around enteric ganglia, which are termed intraganglionic laminar endings and thought to be tension receptors (Berthoud and Neuhuber, 2000; Phillips and Powley, 2000). While vagal afferents do not communicate with enteric neurons to any significant extent (Berthoud et al., 2001), there is pharmacological evidence that transmitters released from spinal sensory nerve fibres act on enteric neurons to influence digestion, notably blood flow, motility and secretory activity (Takaki and Nakayama, 1989; Holzer and Barthó, 1996; Barthó et al., 1999). It thus seems conceivable that spinal afferents feed sensory information into the ENS.

3. Sensory neurons in concert with other gastrointestinal surveillance systems

The abundance of sensory neurons in the gut is very much in place as the gastrointestinal mucosa is the largest external surface extending for an area of 200–300 m^2 in humans. This vast plane is exposed not only to nutrients but also to toxins, antigens and pathogens that come in with the food and to harmful secretory products such as acid and pepsin. In addition, the gastrointestinal tract is home to some 10^{13} bacteria and other microbes which threaten to invade and translocate the gut wall (Autenrieth, 1999). To meet with these demands, the gut is endowed with an elaborate network of surveillance systems that include, besides sensory neurons, immune and enteroendocrine cells (Fig. 2). These sensors act in concert to instigate the appropriate vascular, secretory and motor activities that are necessary to facilitate the uptake of useful contents or to dilute and rapidly expel hazardous materials through diarrhoea and/or emesis.

Enteroendocrine and immune cells are strategically positioned in the gastrointestinal mucosa to analyze the luminal contents. Messenger molecules released from these cells can stimulate adjacent sensory nerve fibres which pass on the information to the ENS and CNS (Fig. 2). Through this interaction, the sensory repertoire of afferent neurons is extended to luminal stimuli that otherwise could not be encoded by their terminals in the lamina propria (Furness and Clerc, 2000). Enteroendocrine cells react to food constituents, gastric acid and bacterial toxins and, in so doing, release neuroactive messengers such as 5-hydroxytryptamine (5-HT), cholecystokinin (CCK), secretin, corticotrophin-releasing factor and somatostatin (Buéno et al., 1997; Lu and Owyang, 1999; Raybould, 1999; Furness and Clerc, 2000).

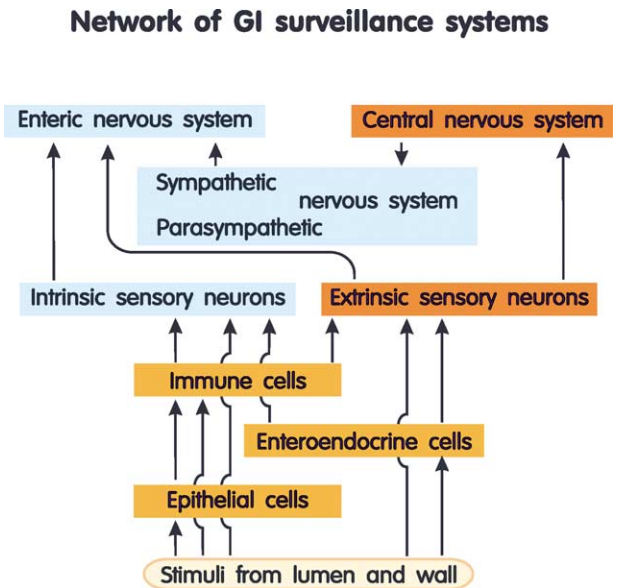

Fig. 2. Gastrointestinal (GI) surveillance systems in concert. These comprise epithelial cells, enteroendocrine cells, immune cells as well as intrinsic and extrinsic sensory neurons which feed their output to the enteric and central nervous system, respectively.

The gastrointestinal immune system includes the gut-associated lymphoid tissue, macrophages, neutrophils, eosinophils, mast cells (Shanahan, 1994; Furness and Clerc, 2000) and chemokine-secreting epithelial cells (Kagnoff and Eckmann, 1997). Whenever the gut is challenged by infection, allergy, inflammation or other injury, the gastrointestinal immune system is called into operation and releases a host of neuroactive mediators (Fig. 2). Among them are cytokines, prostaglandins, leukotrienes, bradykinin, histamine, and serine proteases, all of which can either excite extrinsic afferent nerve fibres, in the short term, or alter their sensitivity, in the long term (Buéno et al., 1997; Furness and Clerc, 2000; Holzer, in press).

4. Peripheral mechanisms of gastrointestinal hyperalgesia

4.1. Gastrointestinal sensation in health and disease

The signals which gastrointestinal sensory neurons convey to the brain are rarely perceived as a conscious sensation because they are processed only in autonomic and neuroendocrine circuits that control digestion in accordance with the body's need of energy, fluid and electrolytes (Mayer, 1995; Wood et al., 1999). This is physiologically meaningful because there are few possibilities to voluntarily control digestive functions, notable exceptions being the conscious reactions to the perception of fullness after a meal (satiety) or the urge to have a bowel movement (Mayer, 1995). Many gastrointestinal afferents, however, have the potential to encode noxious stimuli (Gebhart,

2000), a property that has a bearing on the discomfort and pain associated with functional bowel disorders (De Ponti and Malagelada, 1998; Wood et al., 1999; Drossman et al., 2000). Given that in most cases we do not have a behavioural repertoire with which to appropriately react to abnormal sensations from the gut, gastrointestinal pain is a truly pathological entity.

It is now hypothesized that, in patients with functional bowel disorders, digestive processes are represented in the brain in a distorted fashion, be it because there are pathological alterations in the environment of gut sensors, in the sensory gain of afferent neurons or in the central processing of afferent information from the gastrointestinal tract. Diagnostically, it is obvious that many gut reactions to physiological (e.g., food) and pathological (e.g., stress) stimuli are exaggerated and out of the normal proportion to stimulus strength. This situation is explained by a scenario in which injury, inflammation or anaphylaxis leads to accumulation of neuroactive substances in the gut wall and subsequently to functional, phenotypic and structural alterations in the gastrointestinal innervation. Adaptations of this kind, which may extend far beyond the site of insult and time of original challenge, are thought to underlie the long-lasting disturbances in motor activity, secretory function and visceral sensation typical of functional bowel disorders (Collins, 1996; Spiller et al., 2000).

It has long been held that pain arising from the gastrointestinal tract is mediated exclusively by spinal afferents, whereas the primary task of vagal afferents is to participate in the physiological regulation of digestive activity in the upper gastrointestinal tract (Sengupta and Gebhart, 1994). However, there is now growing awareness that vagal afferents make a distinct contribution to disorder-related alterations in visceral sensation (Gebhart, 2000; Holzer et al., 2000). Thus, vagal sensory neurons respond to a variety of noxious chemicals (Gebhart, 2000), mediate nausea and emesis (Andrews, 1994) and participate in the communication between the peripheral immune system and the CNS (Dantzer et al., 1998; Maier et al., 1998). For instance, the illness responses to infection and inflammation (fever, anorexia, somnolence, decrease in locomotor activity and social exploration and hyperalgesia) are in part signalled by vagal afferents that react to the peripheral generation of interleukin-1β and other proinflammatory cytokines (Dantzer et al., 1998; Maier et al., 1998).

These findings corroborate the view that sensory neurons in the vagus nerve contribute to the emotional-affective aspects of abdominal nociception (Traub et al., 1996). This concept has been borne out by experiments showing that vagal afferents signal a gastric mucosal acid insult to the medullary brainstem (Schuligoi et al., 1998) where the information is passed on to midbrain, thalamic, hypothalamic and limbic nuclei (Michl et al., 2001). There is, however, no activation of the insular cortex, the major cerebral representation area of afferent input from the stomach. Thus, vagal afferent signalling of an acute acid insult in the gastric mucosa does not give rise to perception of pain but leads to activation of subcortical brain nuclei that are involved in emotional, behavioural, autonomic and neuroendocrine reactions to a noxious stimulus (Michl et al., 2001).

4.2. Mechanisms of inflammation-induced gastrointestinal hyperalgesia

The hypothesis that a spell of massive inflammation initiates a persistent change in gastrointestinal sensation comes from reports that gastroenteritis is a major risk factor for developing irritable bowel syndrome (Collins, 1996; Gwee et al., 1999; Spiller et al., 2000). Visceral hypersensitivity involves peripheral and central mechanisms of sensitization, but the factors underlying gastrointestinal allodynia (sensation of pain in response to stimulus strengths that normally are innocuous) and hyperalgesia (exaggerated sensation of pain in response to noxious stimulus strengths) are only in part understood (Buéno et al., 1997; Cervero and Laird, 1999; Gebhart, 2000). Particularly relevant to visceral pain is that most extrinsic afferents innervating the gut have the ability to sensitize (Gebhart, 2000). In addition, the environment of nociceptive nerve terminals in the gut of patients with functional bowel disorders may be profoundly altered, given that the number of enteroendocrine cells, mast cells and mucosal lymphocytes and the permeability of the gastrointestinal mucosa are increased in irritable bowel syndrome (Spiller et al., 2000; O'Sullivan et al., 2000).

Reversible sensitization of nociceptors typically arises from modulation of nerve fibre excitability via post-translational changes such as phosphorylation of receptors, ion channels or associated regulatory proteins (Woolf and Salter, 2000). These processes occur when, following a mucosal insult, peripheral nociceptor terminals are exposed to a mixture of immune and inflammatory mediators including H^+ and K^+ ions, bile salts, ATP, adenosine, histamine, 5-HT, prostaglandins, leukotrienes, platelet-activating factor, serine proteases, cytokines, reactive metabolites and neurotrophic factors (Buéno et al., 1997; Gebhart, 2000). In patients with functional bowel disorders, these modifications of neuronal excitability appear to become permanent and to account for the sensory disturbances that remain after the inflammatory reaction has subsided (Collins, 1996; Gebhart, 2000; Spiller et al., 2000). Convincing evidence for such long-term adaptations has come from the experimental observation that mechanical or chemical irritation of the colon in newborn rats leads to chronic visceral hypersensitivity in the adult animals although no pathology in the colon is discernible (Al-Chaer et al., 2000). Such permanent increases in the sensory gain can be related to changes in the expression of transmitters, receptors and ion channels, changes in the biophysical properties of receptors and ion channels as well as changes in the phenotype, structure, connectivity and survival of

afferent neurons (Woolf and Salter, 2000). These persistent alterations may be instigated by neurotrophic factors that are produced in the inflamed tissue (Lewin, 1996).

4.3. Neuropathy-induced gastrointestinal hyperalgesia

Although peripheral nerve injury produces neurochemical changes in sensory neurons that in many cases are opposite to those occurring after inflammation, the functional result of nerve damage is also hyperalgesia. It awaits to be explored whether neuropathy-associated hypersensitivity is a factor in certain cases of visceral pain, given that pelvic nerve neuritis enhances the mechanosensitivity of afferent nerve fibres (Gebhart, 2000).

5. Molecular targets on sensory neurons for the therapy of functional bowel disorder symptoms and gastrointestinal pain

5.1. Advantages and disadvantages of sensory neuron-targeting drugs

Although there is consensus that both peripheral and central sensitization processes as well as psychological factors contribute to the inappropriate sensations characteristic of functional bowel disorders (Drossman et al., 2000), extrinsic afferents supplying the gut are prime targets at which to aim novel therapies to control gastrointestinal pain (Kirkup et al., 2001). Drugs that act on nociceptive neurons have some advantages over other analgesics, not the least because they hit the first element in the pain pathways. In addition, these drugs may be manufactured such that they cannot enter the brain and hence are free of unwanted adverse effects on CNS functions. Sensory neuron-targeting drugs, though, can also have disadvantages in that they may interfere with the local efferent-like and autonomic reflex functions of primary afferents. Furthermore, they will be ineffective if the pain under treatment is solely the result of central hyperalgesia.

Ideally, sensory neuron-targeting drugs should block the exaggerated signalling of hypersensitive afferents, which implies that they aim at molecular targets that are upregulated in functional bowel disorders (Fig. 3). The complex innervation of the gastrointestinal tract, though, complicates the search for specific traits on extrinsic sensory neurons, and the development of efficacious and safe gastrointestinal analgesics needs to address several key questions.

(i) Which noxious/innocuous stimuli in the gut are relevant to the pain in functional bowel disorders?
(ii) Which receptors/ion channels on extrinsic afferents do these functional bowel disorder-relevant stimuli act on?

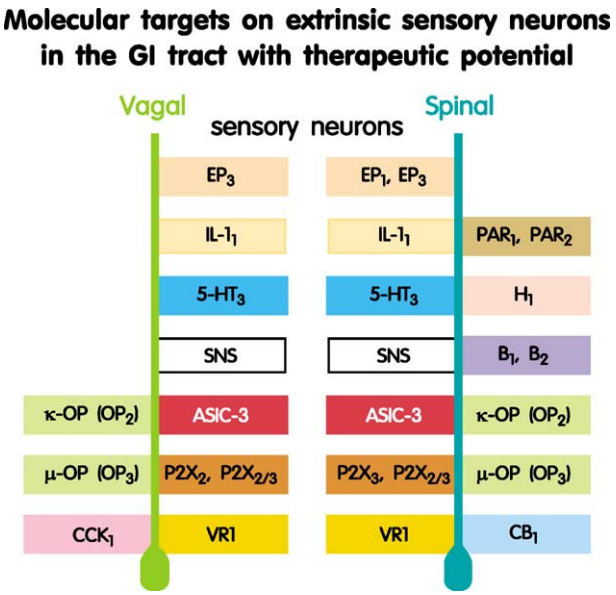

Fig. 3. Selection of sensory neuron-specific molecular targets that could be exploited for the therapy of functional bowel disorders and gastrointestinal (GI) pain. ASIC-3, acid-sensing ion channel type; B_1 and B_2, bradykinin receptor types; CB_1, cannabinoid receptor type; CCK_1, cholecystokinin receptor type; EP_1 and EP_3, prostaglandin E receptor types; H_1, histamine receptor type; 5-HT_3, 5-hydroxytryptamine receptor type; IL-1_1, type 1 interleukin-1 receptor; κ-OP (OP_2) and μ-OP (OP_3), opioid receptor types; PAR-1 and PAR-2, protease-activated receptor types; $P2X_2$, $P2X_3$ and $P2X_{2/3}$, ionotropic purinoceptor types; SNS, sensory nerve-specific Na^+ channel Na_V 1.8; VR1, vanilloid VR1 receptor.

(iii) Which classes of extrinsic afferents do contribute to the pain associated with functional bowel disorders?
(iv) Do the functional bowel disorder-relevant extrinsic afferents express receptors, ion channels or other molecular targets in a cell-specific manner?
(v) Is the expression of pain-relevant targets on extrinsic afferents changed in functional bowel disorders?
(vi) Is drug interference with pain-relevant targets on extrinsic afferents therapeutically efficacious and safe?

Excitatory ion channels such as vanilloid VR1 receptors, acid-sensing ion channels (ASICs), $P2X_3$ purinoceptors and tetrodotoxin-resistant Na^+ channels are of particular relevance because they are selectively expressed by extrinsic afferents (Fig. 3) and, when activated, increase the intracellular Ca^{2+} concentration and thereby stimulate Ca^{2+}-dependent kinases, regulate gene expression and alter the cellular phenotype. For assessing the significance of these targets in visceral hyperalgesia, it is important to know whether number, subunit composition and biophysical properties of sensory neuron-specific ion channels and receptors are persistently altered after a visceral insult (Gebhart, 2000). In addition, targets such as 5-HT, CCK, glutamate, tachykinin, CGRP, γ-aminobutyric (GABA), opioid, cannabinoid, prostaglandin and protease-activated

receptors are also worth exploring (Fig. 3). In developing drugs along these lines, it will be important to assess which quantitative contribution sensory neuron-specific targets make to the induction of hyperalgesia and whether modulation of a single target is therapeutically sufficient.

5.2. Vanilloid receptor ion channels

Owing to its apparently selective actions on primary afferent neurons, the vanilloid capsaicin has long been used as a probe to examine sensory neuron functions in the gastrointestinal tract (Holzer and Barthó, 1996; Holzer, 1998a). The response of afferent neurons to capsaicin consists of two distinct phases: initial excitation followed by a long-lasting refractory state during which the neurons do not respond to capsaicin and other stimuli (Holzer, 1991; Szallasi and Blumberg, 1999). This refractory state is usually reversible after several weeks, unless very high doses of capsaicin are administered systemically to cause permanent neurotoxicity. Various capsaicin analogues differ in the relative ratio of initial excitation to subsequent refractoriness, resiniferatoxin and the capsaicin analogue SDZ 249-665 being examples that hardly stimulate but effectively desensitize sensory neurons (Szallasi and Blumberg, 1999; Urban et al., 2000).

The sensory neuron-selective effects of capsaicin are mediated by the vanilloid VR1 receptor which is a non-selective cation channel with high permeability for Ca^{2+}. Assembled from six transmembrane domains, the vanilloid VR1 receptor is a relative of the transient receptor potential family of store-operated Ca^{2+} channels (Caterina et al., 1997) and homologous to a functionally diverse group of vanilloid VR1 receptor-related proteins (Szallasi and Di Marzo, 2000). The vanilloid VR1 receptor operates as a polymodal detector of potentially harmful stimuli (Fig. 4) including noxious heat, the vanilloid capsaicin, H^+ ions, anandamide and several lipoxygenase products (Caterina et al., 1997; Tominaga et al., 1998; Zygmunt et al., 1999; Hwang et al., 2000; Jordt et al., 2000). While mild acidosis only augments vanilloid VR1 receptor activation by capsaicin and other stimuli, more pronounced acidosis (pH < 6) can per se gate the vanilloid VR1 receptor. Importantly, vanilloid VR1 receptor channel activity is regulated by protein kinase C (Fig. 4), and vanilloid VR1 receptor function is enhanced by sensory neuron stimulants that activate protein kinase C (e.g., bradykinin and anandamide) but are inactive or weakly active agonists at vanilloid VR1 receptors (Premkumar and Ahern, 2000). With these properties, the vanilloid VR1 receptor may encode a variety of chemical stimuli that themselves are unable to directly gate the vanilloid VR1 receptor.

Functional studies indicate that vanilloid VR1 receptor agonists act almost exclusively on primary sensory neurons (Holzer, 1991; Szallasi and Blumberg, 1999), although vanilloid VR1 receptors are expressed both by primary afferent and central neurons (Caterina et al., 1997; Guo et al., 1999a; Michael and Priestley, 1999; Mezey et al., 2000). In rodents, most spinal afferent neurons supplying the gut are sensitive to the sensory neuron-blocking actions of capsaicin (Green and Dockray, 1988; Sternini, 1992), while the proportion of capsaicin-sensitive fibres among the vagal afferents supplying the oesophagus and stomach is minor (Berthoud et al., 1997; Blackshaw et al., 2000) and enteric neurons are insensitive to capsaicin (Holzer, 1991; Holzer and Barthó, 1996). There is ample evidence that capsaicin-induced gating of vanilloid VR1 receptors stimulates extrinsic afferents of the gut (Holzer, 1998a, 1999; Maubach and Grundy, 1999; Su et al., 1999; Blackshaw et al., 2000) and, at excessive concentrations, may give rise to pain in humans (Gonzalez et al., 1998; Hammer et al., 1998; Rodriguez-Stanley et al., 2000). Genetic deletion of the vanilloid VR1 receptor has revealed that sensory neuron responses to capsaicin, protons or noxious heat are suppressed and inflammatory hypersensitivity to heat is attenuated, whereas mechanical nociception remains unaltered (Caterina et al., 2000; Davis et al., 2000). However, visceral sensitivity has not yet been tested in vanilloid VR1 receptor-deficient mice.

The apparently selective association of vanilloid VR1 receptors with extrinsic afferents of the gut makes this cation channel an intriguing focus for drug development. Although acid challenge in the rat stomach and duodenum seems to activate vanilloid VR1 receptors (Holzer, 1998a; Akiba et al., 1999), it awaits to be revealed which other stimuli and conditions in the gastrointestinal tract act on vanilloid VR1 receptors and whether vanilloid VR1 receptors are important transducers of gut sensation. Since the

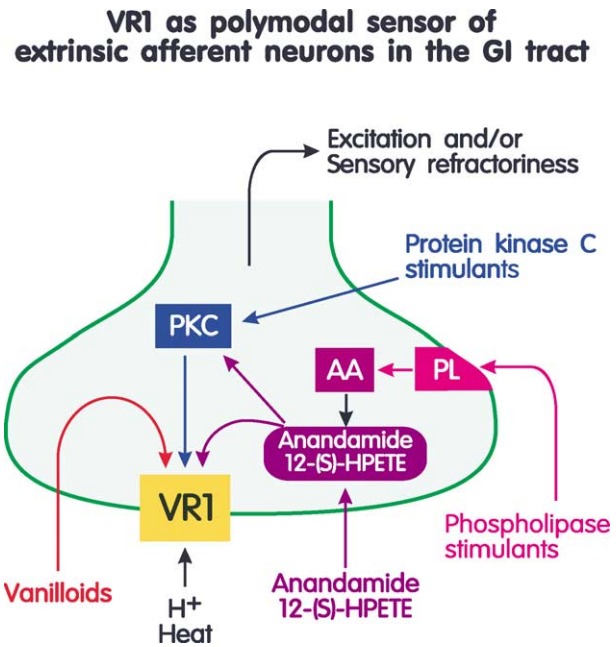

Fig. 4. The vanilloid VR1 receptor as a polymodal sensor of noxious stimuli and its regulation by protein kinase C (PKC). AA, arachidonic acid; GI, gastrointestinal; PL, phospholipase; 12-(*S*)-HPETE, 12-(*S*)-hydroperoxyeicosatetraenoic acid.

expression of vanilloid VR1 receptors is regulated by neurotrophic factors (Winter, 1998) and their activity augmented by inflammatory mediators (Premkumar and Ahern, 2000), blockade of vanilloid VR1 receptors may be an efficacious way to suppress visceral hyperalgesia. While the vanilloid VR1 receptor antagonist capsazepine has been tested only in models of somatic hyperalgesia (Kwak et al., 1998), there is information that vanilloid VR1 receptor-mediated sensory neuron refractoriness is effective in visceral hypersensitivity. Thus, resiniferatoxin reverses urinary bladder hyperreflexia in humans (Silva et al., 2000) and SDZ 249-665 attenuates inflammatory bladder hyperreflexia, referred hyperalgesia (Jaggar et al., 2001) and behavioural pain responses (abdominal muscle constrictions, often termed "visceromotor responses") to intraperitoneal acetic acid in rats (Urban et al., 2000).

5.3. Acid-sensing ion channels

ASICs comprise a family of proton-gated Na^{2+} channels that are made up of different subunits some of which (e.g., ASIC-1b and ASIC-3) are exclusively expressed in the cell membrane of primary afferent neurons (Chen et al., 1998; Waldmann et al., 1999). These channels play a role not only in taste transduction but also in nociception, given that the pH in inflamed tissue may be lowered to a considerable extent and acidosis is known to sensitize and activate nociceptive afferent neurons (Reeh and Steen, 1996). Within the gut, it is well conceivable that ASICs mediate some of the sensory neuron responses to acid challenge in the gastroduodenal mucosa, an implication that needs yet to be tested.

5.4. P2X purinoceptor ion channels

P2X purinoceptors are ligand-gated cation channels that are assembled from different subunits, seven of which have been identified at the molecular level (North and Surprenant, 2000; Burnstock, 2001). The P2X receptors on vagal afferents comprise homomeric $P2X_2$ and heteromeric $P2X_{2/3}$ receptors whereas those on spinal afferents are of the homomeric $P2X_3$ and heteromultimeric $P2X_{2/3}$ type (Fig. 5; Chen et al., 1995; Lewis et al., 1995; Burnstock, 2001). Since in the gut ATP can be released from enteric, sympathetic and extrinsic afferent neuerons, epithelial, endothelial and immune cells as well as by cell damage of any kind (Fig. 5; Barthó et al., 1999; Burnstock, 2001; Di Virgilio et al., 2001), the extracellular purine concentrations may be elevated under a variety of physiological and pathological circumstances. Following pepsin-induced inflammation of the ferret oesophagus, ATP is able to sensitize vagal afferents to mechanical stimuli (Page et al., 2000). Mesenteric afferents supplying the proximal rat small intestine are activated by ATP both via a direct action on the nerve fibres and via ATP-induced motor changes (Kirkup et al., 1999).

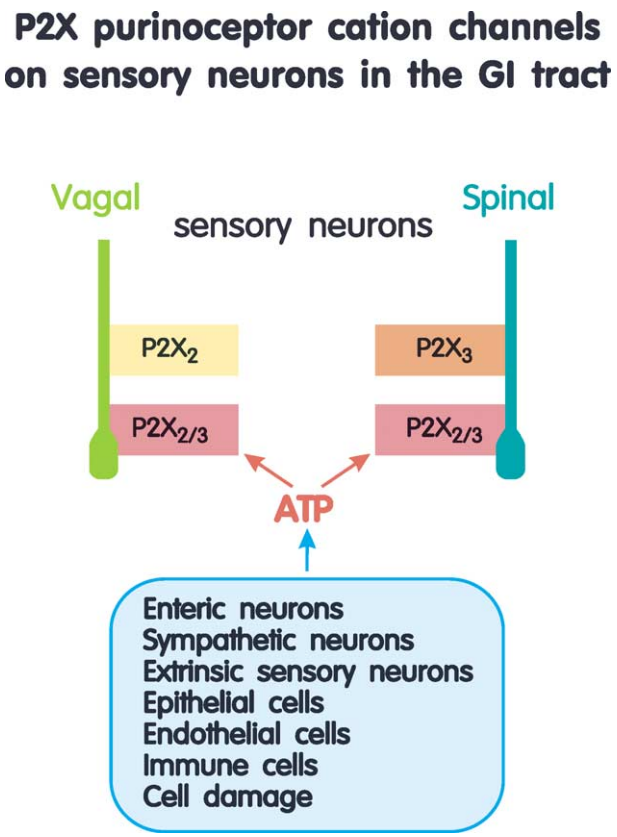

Fig. 5. Possible sources of extracellular adenosine triphosphate (ATP) in the gastrointestinal (GI) tract and its targets of action (P2X purinoceptors) on extrinsic afferent nerve fibres supplying the gut.

Since the $P2X_3$ receptor type is exclusively expressed by primary afferent neurons that are thought to subserve a nociceptive function (Chen et al., 1995; Lewis et al., 1995; Burnstock, 2001), blockade of these purinoceptors is considered to selectively target pain pathways. This implication extends to visceral nociception as shown by the finding that genetic deletion of $P2X_3$ receptors causes urinary bladder hyporeflexia (Cockayne et al., 2000). Whether pharmacological inhibition of $P2X_3$ receptors attenuates sensory neuron responses to distension and tissue damage in the gut awaits to be shown. As other P2X receptor subtypes are also expressed by enteric neurons and intestinal muscle (Galligan et al., 2000), it need be kept in mind that nonselective blockade of these purinoceptors will interfere with ENS function (Galligan et al., 2000) and peristaltic motor regulation (Heinemann et al., 1999a).

5.5. Sensory neuron-specific Na^+ channels

The family of voltage-gated Na^{2+} channels includes two tetrodotoxin-resistant members, SNS/PN3 (Na_V 1.8) and SNS2/NaN (Na_V 1.9), that are specifically located on spinal and vagal afferents (Bielefeldt, 2000; Baker and Wood, 2001). Inflammatory mediators enhance tetrodoto-

xin-resistant voltage-dependent Na^+ currents (England et al., 1996) and inflammation leads to increased expression of SNS/PN3 Na^{2+} channels in sensory neurons (Okuse et al., 1997). While there is experimental evidence that SNS/PN3 Na^{2+} channels are involved in somatic hyperalgesia (Akopian et al., 1999; Porreca et al., 1999), any role of tetrodotoxin-resistant Na^+ channels in visceral pain has not yet been addressed.

5.6. Mechanosensitive ion channels

The ability of gastrointestinal afferent neurons to detect stretch, contraction or other mechanical deformations of the gut wall is related to the expression of specific mechanosensors one of which, a mechanosensitive K^+ channel, has been characterized in sensory neurons supplying the rat colon (Su et al., 2000). It awaits to be evaluated whether pharmacological interference with such mechanosensors would be of benefit to those patients with functional bowel disorders in whom visceral discomfort is triggered by hypersensitive afferents that respond to physiological alterations of gut motility in an exaggerated manner. Any antinociceptive effect of mechanosensor blockers will have to be carefully judged against their possible influence on mechanically triggered motor and secretory reflexes whereby the ENS regulates digestion.

5.7. 5-HT_3 and 5-HT_4 receptors

5-HT exerts many actions on enteric neurons and extrinsic afferents of the gut, which is related to the expression of multiple 5-HT receptor types by these neurons. The fact that 5-HT_3 receptors abound on extrinsic sensory neurons has stimulated efforts to target these receptors in an attempt to control the nausea, discomfort and pain associated with functional bowel disorders (Buéno et al., 1997; De Ponti and Malagelada, 1998; Camilleri, 2001a). This concept has been successfully borne out by the development of 5-HT_3 receptor antagonists as antiemetic drugs. Released from enterochromaffin (EC) cells by cisplatin and related cytostatic compounds, 5-HT stimulates 5-HT_3 receptors on vagal afferents involved in the emetic reflex, an effect that is suppressed by 5-HT_3 receptor antagonists (Andrews, 1994). 5-HT_3 receptors are also expressed by spinal sensory neurons that are involved in the afferent signalling of colorectal distension (Kozlowski et al., 2000). Accordingly, the 5-HT_3 receptor antagonist alosetron has been reported to reduce discomfort and pain in female patients suffering from functional dsypepsia or diarrhoea-predominant irritable bowel syndrome (Bardhan et al., 2000; Talley et al., 2001). Since 5-HT_3 receptors are also present on several groups of enteric neurons (Bertrand et al., 2000), the use of 5-HT_3 receptor antagonists as antinociceptive drugs in functional bowel disorders is limited by their inhibitory action on peristalsis and fluid secretion, resulting in constipation.

Of the 5-HT_4 receptor agonists tested on humans, the "prokinetic" cisapride acts preferentially on the proximal gastrointestinal tract and is beneficial in gastro-oesophageal reflux, gastroparesis and functional dyspepsia (Briejer et al., 1995). The "enterokinetic" 5-HT_4 receptor agonists prucalopride and tegaserod enhance propulsion preferentially in the small and large intestine and help alleviating functional constipation (Bouras et al., 1999) and constipation-predominant irritable bowel syndrome (Camilleri, 2001b). Since visceral sensation in healthy humans is not affected by 5-HT_4 receptor antagonism (Bharucha et al., 2000), it appears that the beneficial action of 5-HT_4 receptor agonists in the gut is primarily related to an improvement of ENS function. The eventual place of 5-HT_4 receptor agonists in the therapy of constipation-predominant irritable bowel syndrome and the mechanism of their pain-relieving action await to be established.

5.8. CCK_1 receptors

CCK_1 receptors on vagal afferents (Sternini et al., 1999) may be relevant targets for the treatment of functional dyspepsia, because CCK_1 receptor antagonists can attenuate the meal-like fullness and nausea associated with intraduodenal lipid and gastric distension (Feinle et al., 2001). Whether CCK_1 receptor antagonists are beneficial in irritable bowel syndrome is not known (Buéno et al., 1997) but may seem worth testing given that in irritable bowel syndrome patients CCK causes exaggerated motor responses and abdominal pain (Roberts-Thomson et al., 1992).

5.9. Somatostatin receptors

The somatostatin analogue, octreotide, reduces the perception of gastric and rectal distension in healthy volunteers and irritable bowel syndrome patients (Bradette et al., 1994; Chey et al., 1995; Mertz et al., 1995). Whether this effect of octreotide is due to stimulation of distinct somatostatin receptors on extrinsic afferents awaits to be evaluated.

5.10. Bradykinin receptors

Bradykinin is formed, e.g., in the acid-threatened gastric mucosa (Pethö et al., 1994) and during mesenteric ischaemia (Guo et al., 1999b) and is likely to act on gastrointestinal afferents, since intraperitoneal bradykinin elicits cardiovascular and gastric reflex responses (Holzer-Petsche and Brodacz, 1999) as well as behavioural signs of pain (Heapy et al., 1993). These reactions are brought about by activation of bradykinin B_2 receptors and are related to the kinin's ability to stimulate serosal afferents from the intestine (Guo et al., 1999b; Maubach and Grundy, 1999). Bradykinin receptor antagonists could help reducing visceral hyperalgesia, because the bradykinin B_2 receptor

antagonist icatibant counteracts the inflammation-induced increase in the abdominal constriction responses to colorectal distension and intraperitoneal acetic acid (Julia et al., 1995). In a model of nematode-induced infection of the intestine it has been shown that both a bradykinin B_1 and a bradykinin B_2 receptor antagonist can attenuate the post-infection hypersensitivity to jejunal distension (McLean et al., 1998b). Likewise, the hyperreflexia of the urinary bladder in an experimental cystitis model involves bradykinin B_1 and B_2 receptors, with bradykinin B_1 receptors coming into play only after inflammation has set in (Jaggar et al., 1998a).

5.11. Prostaglandin receptors

The most widely used antinociceptive drugs are nonsteroidal antiinflammatory drugs whose major adverse effect, though, is damage to the gastrointestinal mucosa. Although this effect seems to arise primarily from inhibition of the cyclooxygenase isoform cyclooxygenase-1 (Warner et al., 1999), it awaits to be examined whether selective cyclooxygenase-2 inhibitors which largely spare the gastroduodenal mucosa (Laine, 2001) have any therapeutic potential in functional bowel disorders. Prostaglandin receptors are further important targets, given that prostaglandins including prostaglandin E_2 and prostaglandin I_2 are key mediators of hyperalgesia and primary sensory neurons express prostaglandin EP_1, EP_3, EP_4 and IP receptors (Bley et al., 1998; Ek et al., 1998; Nakamura et al., 2000). Accordingly, prostaglandin E_2 excites mesenteric afferent nerve fibres supplying the rat intestine (Haupt et al., 2000) and sensitizes visceral afferents to other algesic substances (Maubach and Grundy, 1999) including bradykinin, an action that seems to be brought about by prostaglandin EP_3 receptors (Kumazawa et al., 1996). These circumstances suggest that prostaglandin receptor antagonists are worthwile alternatives to cyclooxygenase inhibitors in the treatment of visceral pain and hyperalgesia.

5.12. Protease-activated receptors

Serine proteases such as mast cell tryptase, trypsin and thrombin can stimulate a particular group of cell surface receptors termed protease-activated receptors (PARs). Of the 4 PARs identified thus far, PAR-1 and PAR-2 are expressed by dorsal root ganglion neurons (Vergnolle et al., 2001), although in the gut these receptors are also found on distinct classes of enteric neurons (Corvera et al., 1999; Green et al., 2000). Administration of a PAR-2 agonist excites afferent axons in jejunal mesenteric nerves of the rat and causes a delayed hypersensitivity to colorectal distension (Vergnolle et al., 2001). It awaits to be explored whether PAR-1 or PAR-2 antagonists are of any utility in the control of visceral hyperalgesia.

5.13. Glutamate receptors

Glutamate occurs in most vagal and spinal sensory neurons (Schaffar et al., 1997; Keast and Stephensen, 2000) and contributes to the transmission between primary and secondary afferents in the CNS via action on ionotropic NMDA (*N*-methyl-D-aspartate), AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate receptors as well as group I metabotropic receptors of subtype 1 and 5 (Foley et al., 1998; Fundytus et al., 1998; Kessler and Baude, 1999; Zhai and Traub, 1999; Kozlowski et al., 2001). Ionotropic receptors of the NMDA, AMPA and kainate type (Carlton and Coggeshall, 1999) and group I metabotropic glutamate receptors of subtype 5 (Walker et al., 2001) are also found on peripheral terminals of somatic primary afferents. Both NMDA and non-NMDA glutamate receptors are involved in the spinal input evoked by noxious colorectal distension in rats (Zhai and Traub, 1999; Kozlowski et al., 2001). Within the brainstem, NMDA receptors participate in the transmission of gastric distension in rats (Zheng et al., 1999) and non-NMDA ionotropic glutamate receptors contribute to cisplatin-induced emesis in ferrets (Fink-Jensen et al., 1992).

In contrast, the afferent signalling of an acute gastric mucosal acid insult to the rat brainstem is not affected by NMDA and non-NMDA glutamate receptor antagonists (Jocic et al., 2001). When, however, the stomach is repeatedly injured by acid, a role of NMDA receptors in the communication from the acid-threatened stomach to the brainstem becomes obvious (Jocic et al., 2001). A similar enhancement of NMDA receptor function in the spinal cord is seen following repeated colorectal distension, a condition that induces inflammation (Zhai and Traub, 1999). However, the therapeutic utility of glutamate, particularly NMDA, receptor antagonists as analgesics has remained limited because of their adverse effects on CNS function (Fisher et al., 2000). It awaits to be explored whether antagonists targeting other glutamate receptors or glutamate receptor antagonists with a peripherally restricted site of action will prove useful in visceral pain states.

5.14. Tachykinin and CGRP receptors

Since the vast majority of spinal afferents supplying the rodent gastrointestinal tract expresses CGRP and the tachykinin substance P (Perry and Lawson, 1998), antagonists of these peptide transmitters are explored for their therapeutic potential in irritable bowel syndrome (Camilleri, 2001a). Although this application may be limited by the presence of substance P and CGRP in the ENS and their role in the neural regulation of gastrointestinal motility, secretion and circulation (Holzer and Holzer-Petsche, 1997a,b; Holzer, 1998b), experimental evidence points to a role of these peptides in gastrointestinal pain. In tachykinin

NK_1 receptor-deficient mice, intracolonic administration of acetic acid and capsaicin fails to elicit cardiovascular responses indicative of pain, whereas the reaction to distension is normal (Laird et al., 2000). Importantly, tachykinin NK_1 receptor knockout prevents intracolonic acetic acid and capsaicin from inducing primary mechanical hyperalgesia in the colon and referred mechanical hyperalgesia in the abdominal skin (Laird et al., 2000).

Behavioural pain responses to colorectal distension in the rat are attenuated by a tachykinin NK_2 and NK_3 receptor antagonist but left unaltered by a tachykinin NK_1 receptor antagonist and the CGRP receptor antagonist CGRP-(8-37) (Plourde et al., 1997; Julia et al., 1999). In contrast, the mechanical hyperalgesia induced by colonic inflammation due to acetic acid instillation is blocked by the CGRP receptor antagonist (Plourde et al., 1997). The pain response to intraperitoneal acetic acid is likewise depressed by CGRP-(8-37) and a tachykinin NK_2, but not NK_1, receptor antagonist (Julia and Buéno, 1997), while the cardiovascular responses to peritoneal irritation or jejunal distension are susceptible to inhibition by a tachykinin NK_1 and NK_2, but not NK_3, receptor antagonist (Holzer-Petsche and Rordorf-Nikolić, 1995; McLean et al., 1998a). Furthermore, tachykinin NK_2 receptors mediate the jejunal hypersensitivity that develops in rats infected with *Nippostrongylus brasiliensis* (McLean et al., 1997).

The precise sites at which tachykinin and CGRP receptors mediate visceral pain are not known. Since irritation, immune challenge and inflammation releases CGRP and substance P from both extrinsic afferents and intrinsic enteric neurons within the gut (Friese et al., 1997; Holzer, 1998a), these peptides may either increase the peripheral sensory gain of extrinsic afferents within the gut or contribute to primary afferent transmission within the CNS (Buéno et al., 1997; Maggi, 1997). As extrinsic afferents of the gut do not seem to possess receptors for tachykinins and CGRP, it is very likely that peptide-evoked sensitization or excitation of sensory neurons is an indirect consequence of peptide actions on gastrointestinal effectors (Maggi, 1997; Holzer, 1998b; McCarson, 1999; Laird et al., 2000). For instance, tachykinins play a role in the intestinal hypermastocytosis, hypersecretion and motor disturbance evoked by anaphylaxis (Fargeas et al., 1993; Gay et al., 1999), and in nematode-infected rats the tachykinin NK_2 receptor-mediated hypersensitivity to intestinal distension is confined to those areas in which the number of mast cells is increased (McLean et al., 1997).

The experimental evidence thus suggests that tachykinin NK_1 and NK_2 receptor antagonists are of therapeutic utility in functional bowel disorders. If tachykinin NK_1 receptor antagonists are manufactured such that they cross the blood–brain barrier, their antiemetic (Diemunsch and Grelot, 2000), anxiolytic and antidepressive (Kramer et al., 1998) activities may favourably combine with their analgesic properties. CGRP receptor antagonists may also have great potential, although the implication of this peptide in visceral pain states is still little known. It must not be overlooked, though, that the analgesic efficacy of peptide receptor antagonists may be limited because CGRP and substance P are only cotransmitters of glutamatergic sensory neurons. In addition, glutamate and substance P cooperate in a complex manner such that in the spinal cord activation of presynaptic NMDA receptors facilitates transmitter release from afferent nerve endings (Liu et al., 1997; Malcangio et al., 1998), while activation of postsynaptic tachykinin NK_1 receptors enhances NMDA receptor excitability via a protein kinase C pathway (Urban et al., 1994; Wajima et al., 2000; Zou et al., 2000). A similar gating of NMDA receptor channels through activation of tachykinin NK_1 receptors in the brainstem (Liu et al., 1998) explains why the vagal afferent signalling of an acute gastric acid insult is blocked only when a NMDA receptor antagonist is combined with a tachykinin NK_1 and NK_2 receptor antagonist (Jocic et al., 2001). It need hence be kept in mind that effective blockade of afferent transmission in the spinal cord and brainstem may be achieved only by combined antagonism of the major cotransmitters of primary sensory neurons.

5.15. $GABA_B$ receptors

Both $GABA_A$ and $GABA_B$ receptors are expressed by vagal and spinal sensory neurons (Ashworth-Preece et al., 1997; Rusin and Moises, 1998; Marvizon et al., 1999), but only $GABA_B$ receptor activation inhibits the release of substance P from afferent nerve endings in the spinal cord (Malcangio and Bowery, 1993; Marvizon et al., 1999; Riley et al., 2001). As a result, the $GABA_B$ receptor agonist baclofen exerts antinociceptive effects in the spinal cord (Dirig and Yaksh, 1995) and inhibits vagal afferent input from the stomach to the brainstem (Yuan et al., 1998). Further analysis has shown that activation of both peripheral and central $GABA_B$ receptors inhibits distension-sensitive, but not chemosensitive, gastro-osophageal afferents in the vagus nerve (Page and Blackshaw, 1999; Partosoedarso et al., 2001). These actions are likely to have a bearing on the ability of baclofen to prevent gastro-oesophageal reflux (Lidums et al., 2000; Staunton et al., 2000), whereas a possible influence of $GABA_B$ receptor agonists on gastrointestinal pain has not yet been addressed.

5.16. α_2-Adrenoceptor agonists

Spinal afferents express α_2-adrenoceptors which in the human dorsal root ganglia are preferentially of the α_{2B} and α_{2C} subtype (Birder and Perl, 1999; Ongjoco et al., 2000). It is by stimulation of these presynaptic receptors that noradrenaline can inhibit the release of substance P and glutamate from afferent nerve terminals in the spinal cord and thus interfere with transmission at the first synapse of nociceptive pathways (Ono et al., 1991; Ueda et

al., 1995; Fürst, 1999; Millan, 1999). Such a mode of action could have a bearing on the ability of the α_2-adrenoceptor agonist clonidine to reduce the sensation and discomfort associated with gastric and colorectal distension (Thumshirn et al., 1999; Malcolm et al., 2000). The effect of tricyclic and other antidepressant drugs to ameliorate the pain associated with functional bowel disorders (Cannon et al., 1994; Tanum and Malt, 1996; Camilleri, 2001a) may also be in part related to extracellular accumulation of noradrenaline and subsequent stimulation of α_2-adrenoceptors (Sawynok and Reid, 1992; Su and Gebhart, 1998; Gray et al., 1999).

5.17. Peripheral opioid receptors

Opioid receptors on nociceptive afferent neurons have attracted considerable interest because activation of these receptors by drugs that do not enter the brain may afford analgesia without adverse effects on CNS function. Both spinal and vagal sensory neurons express different numbers of μ-, δ- and κ-opioid receptors which are transported into the peripheral axons (Ji et al., 1995; Minami et al., 1995; Rusin and Moises, 1998; Aicher et al., 2000). Because of drug dependence considerations, most attention has been devoted to peripheral κ-opioid receptors, although stimulation of peripheral μ- and δ-opioid receptors can also inhibit visceral nociception (Craft et al., 1995; Reichert et al., 2001). Distension-induced discharges in pelvic and vagal afferents are inhibited by intraarterial as well as intraluminal administration of κ-opioid receptor agonists such as asimadoline, spiradoline and fedotozine, but left unaltered by μ- and δ-opioid receptor agonists (Sengupta et al., 1999; Ozaki et al., 2000). Trinitrobenzene sulphonic acid-induced inflammation of the colon enhances the potency of κ-opioid receptor agonists to inhibit distension-evoked activity in pelvic afferents (Sengupta et al., 1999). The analgesic effect of κ-opioid receptor agonists and the apparent upregulation of peripheral κ-opioid receptors in colonic inflammation is also seen when behavioural pain responses to colonic distension are studied (Sengupta et al., 1999).

The identity of the receptors involved in the peripheral analgesic effect of κ-opioid receptor agonists is in dispute. Since the antinociceptive action of asimadoline and spiradoline is only modestly inhibited by nor-binaltorphimine, it is thought that these compounds act via non-κ-opioid receptors, an inference that is supported by the results of κ-opioid receptor knockdown experiments (Joshi et al., 2000). In contrast, the effects of fedotozine to inhibit spinal c-*fos* expression in response to intraperitoneal administration of acetic acid and to reverse the enhanced visceromotor response to distension of the inflamed rat colon are attenuated by nor-binaltorphimine (Langlois et al., 1997; Bonaz et al., 2000). Fedotozine also exhibits some clinical activity as it increases the threshold at which gastric distension causes pain in healthy volunteers (Coffin et al., 1996), relieves some symptoms associated with functional dyspepsia (Read et al., 1997) and enhances the threshold at which colonic distension gives rise to discomfort in irritable bowel syndrome patients (Delvaux et al., 1999). As compliance of the gastrointestinal wall is not altered by fedotozine in these studies, it appears as if this drug is antinociceptive through an action on afferent neurons. However, the definite efficacy and safety of κ-opioid receptor agonists in the treatment of functional bowel disorders is not yet known, and it has to be kept in mind that these compounds do not differ from μ-opioid receptor agonists in suppressing enteric nerve activity and propulsive motility (Waterman et al., 1992; Allescher et al., 2000).

Apart from inhibiting sensory nerve activity, low doses of μ- and δ-opioid receptor agonists can excite vagal afferents (Eastwood and Grundy, 2000). It awaits to be proved whether this opioid action is related to the ability of certain capsaicin-sensitive vagal afferents to activate descending pathways in the CNS and thereby to inhibit spinal nociception (Randich and Gebhart, 1992; Ren et al., 1993). The spectrum of apparently paradox opioid actions on the vagal afferent system is extended by the observation that fedotozine enhances the afferent input from the acetic acid-irritated peritoneum to the brainstem (Bonaz et al., 2000).

5.18. Peripheral cannabinoid receptors

Cannabinoid CB_1 receptors are present on dorsal root ganglion neurons that express vanilloid VR1 receptors but hardly contain substance P or CGRP (Ahluwalia et al., 2000). Synthesized in the somata, cannabinoid CB_1 receptors are transported not only to the central but also to the peripheral terminals of sensory neurons (Hohmann and Herkenham, 1999), where they are thought to interfere with nerve excitation by noxious stimuli (Piomelli et al., 2000; Szallasi and Di Marzo, 2000). Within the gut, cannabinoid CB_1 receptors have also been localized to the ENS (Kulkarni-Narla and Brown, 2000), and stimulation of these receptors inhibits the release of acetylcholine from enteric neurons, suppresses peristaltic motility and exerts an anti-diarrhoeal effect (Pertwee, 1997; Heinemann et al., 1999b; Izzo et al., 1999). These effects of CB_1 receptor agonists must be kept in mind when CB_1 receptors on extrinsic afferents are considered as targets of antinociceptive drugs. There is in fact evidence that somatic tissue damage may cause formation and release of endogenous cannabinoids (endocannabinoids) such as anandamide, which via cannabinoid CB_1 receptor activation block the stimulation of sensory nerve fibres by the noxious event (Calignano et al., 1998). A similar process takes place on pelvic afferents, whereby anandamide prevents and reverses inflammation-associated hyperreflexia of the urinary bladder (Jaggar et al., 1998b). However, an implication of endocannabinoids in gastrointestinal hypersensitivity has not yet been explored.

6. Concluding remarks

Efforts to identify molecular traits that are selectively expressed by sensory neurons have come up with a number of receptors and ion channels that hold considerable potential for therapeutic exploitation in functional bowel disorders. It is now important to explore whether the number and/or behaviour of these sensory neuron-specific targets is altered in persistent states of pain and whether pharmacological manipulation of such traits can correct gastrointestinal hypersensitivity. Several attempts to treat symptoms of functional bowel disorders with, e.g., 5-HT_3 receptor antagonists or κ-opioid receptor agonists have suffered from a low efficacy relative to a high rate of placebo response. Another point that need to be accounted for is that a number of transmitter receptors expressed by extrinsic afferents is also found on enteric neurons and that interference with these messenger systems may compromise normal digestive functions.

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