Pathobiology of Visceral Pain: Molecular Mechanisms and Therapeutic Implications

III. Visceral afferent pathways: a source of new therapeutic targets for abdominal pain*

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Buéno, Lionel, Jean Fioramonti, and Rafael Garcia-Villar. Pathobiology of Visceral Pain: Molecular Mechanisms and Therapeutic Implications. III. Visceral afferent pathways: a source of new therapeutic targets for abdominal pain. Am J Physiol Gastrointest Liver Physiol 278: G670–G676, 2000.—Visceral pain is the major cause of consulting in gastroenterology and the principal symptom of functional bowel disorders. This symptom is often associated with gut hypersensitivity to distension. The use of animal models has recently permitted the identification of some mediators supposed to play a pivotal role in the genesis of visceral hypersensitivity. Serotonin, through different receptor subtypes, as well as kinins and calcitonin gene-related peptide, are known to be involved, but other putative transmitters arise and are new potential targets for the development of efficacious treatments. This themes article addresses both physiological and preclinical issues of interest for the selection of active new drugs in regard to the clinical pharmacology of visceral pain.

gut; mediators; hypersensitivity; functional bowel disorders; serotonin; bradykinin; tachykinins; N-methyl-D-aspartate; calcitonin gene-related peptide

ABDOMINAL PAIN may result from visceral organic disease or surgery, but it also represents the major symptom of functional bowel disorders (FBD) such as non-ulcer dyspepsia or irritable bowel syndrome (IBS). In contrast to other chronic pain diseases, visceral pain is often associated with gut motor abnormalities that generate exaggerated intraluminal pressures. Clinical observations in IBS patients indicate that motor functional reflexes are enhanced and associated with a lowering of the intraluminal pressure generating pain sensations. These observations are in agreement with electrophysiological data in animals showing that, under inflammatory conditions, both low- and high-threshold baroreceptors of the gut wall are activated at lower, normally ineffective, intraluminal pressures. Compared with somatic nociceptive signal generation, the presence in the gut wall of silent nociceptors that can be activated by immune signals also seems to be important in the genesis of chronic abdominal pain. At the gut level, there are also specific connections between the immune system and intrinsic and extrinsic innervation that modulate local immune and functional reactions.

During the past few years, special attention has been paid to the specific roles of mast cells and cytokines. Both are able to induce long-term changes in signaling to the brain and play a pivotal role in the alteration of motor reflexes and barosensitivity associated with gastrointestinal symptoms. This themes article focuses on current knowledge of the structures and mediators specifically involved at the gastrointestinal tract level in experimental situations that attempt to mimic events in visceral hyperalgesia.

SENSITIZATION OF PRIMARY AFFERENTS VS. DORSAL HORN

Peripheral sensitization of primary afferent neuron terminals within the gut results in a decrease in the intensity and/or amplitude of the stimulus required to initiate their depolarization and in an increase in the number and/or amplitude of neuronal discharges in response to a given chemical or mechanical stimulus. This peripheral sensitization is believed to result from the release of proinflammatory substances at the site of injury, such as bradykinin, tachykinins, prostaglandins, serotonin, ATP, and protons (for review, see Refs. 3 and 11). Most of these mediators are known to be algogenic substances that act directly on receptors located at sensory nerve terminals to depolarize these neurons and initiate nociceptive inputs to the spinal cord; they can also lower the threshold for activation by other mediators.

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normally active mechanical and chemical stimuli, and they can activate local immunocytes and/or other cells, like mast cells or sympathetic varicosities, which in turn release algogenic substances acting on sensory nerve endings (Fig. 1). Growth factors, such as nerve growth factor (NGF), are present both in gut tissues and mast cells and are released during mast cell degranulation. These factors are involved in neuronal plasticity but may also change the distribution of receptors to algogenic mediators and the threshold of sensitivity to mechanical and chemical stimuli. In addition, an enhanced expression of sodium channels has been proposed to explain the hypersensitivity of peripheral neurons that results from injury (13).

The sustained activity of primary afferent fibers that occurs after peripheral sensitization favors the release of neuromediators, increasing the efficacy of synaptic transmission between primary afferents and dorsal horn neurons, a process, referred to as central sensitization (for review, see Ref. 9), that involves specific pre- and postsynaptic receptors (Fig. 2). Despite a different localization in laminae between somatic and visceral projecting neurons at the dorsal horn, the mechanisms of sensitization at this level are similar, yet only very few experimental data are available for the gut. The mechanisms that underlie central sensitization are not fully understood. In vitro and in vivo pharmacological studies implicate a cooperation between substance P (SP) and N-methyl-D-aspartate (NMDA)-mediated events in the development and maintenance of inflammation-induced central sensitization (for review, see Ref. 11). The increased responsiveness of dorsal horn neurons in chronic inflammation is largely mediated by activated NMDA receptors. This activation of NMDA receptors depends on protein kinase C activation, particularly of extracellular signal-regulated kinase phosphorylation (14). The interaction of SP receptors with protein kinase C induces the phosphorylation of NMDA receptors, counteracting the magnesium block and allowing NMDA receptors to operate at a more negative potential (for review, see Ref. 19). All of these data strongly suggest that SP and neurokinin (NK)1 receptors are crucial for the induction of central sensitization in rodents. However, the failure of NK1 receptor antagonists in clinical trials for pain states indicates that another receptor may probably fulfill this function in humans. Obvious candidates in spinal cord are the other tachykinin receptors, i.e., NK2 and NK3 receptors (see SPECIFIC MEDIATORS OF GUT HYPERSENSITIVITY). Increased release of these mediators is concomitantly observed at the periphery in visceral inflammation, but the role of SP in sensitization following colonic inflammation remains controversial.

It has recently been shown that neurons expressing high-affinity (TrkA) receptors for NGF participate in the first central relay of transmission of nociceptive information to supraspinal centers, with an increased number of TrkA receptors or TrkA-immunoreactive neurons as demonstrated in chronic pain states (23).

The expression of sodium channels is also enhanced at the spinal cord level in models of neuropathic or inflammation-induced pain. This increase is rapid, prolonged, and well correlated with the hyperalgesia (13), but, in contrast to neuropathic pain, its relevance to inflammation-induced pain remains to be clinically demonstrated using selective sodium channel blockers. The expression of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide is also altered during experimental inflammation and neuropathy [chronic constriction injury (CCI)]-induced chronic pain, suggesting their involvement at the dorsal horn level. Vasoactive intestinal peptide overexpression lasts several weeks, whereas the expression of vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating polypeptide (PACAP) receptor isoforms 1 and 2 (VPAC1, VPAC2) and PACAP type 1 receptor (PAC1) is differently affected according to the experimental model and the lamina considered (10).
MODELS OF GUT HYPERALGIESIA

During the past decade, numerous models have been developed, mainly in rats, to investigate new targets for the treatment of visceral pain. Most of them are based on the induction of either visceral or somatic responses to a local mechanical stimulus. Recently, several models of inflammation-induced or chemically induced pain have been developed to evaluate spontaneous pain or behavioral changes as markers of pain or discomfort. According to the multifactorial origin of pain in FBD and the lowered threshold of pain perception in these patients, local distension of the upper or lower part of the gut is performed. Intraluminal or intraparietal administration of irritating agents such as trinitrobenzenesulfonic acid (TNBS), formalin, or acetic acid (for review, see Ref. 3) often induces hypersensitivity to distension. Other models, related to postinfection visceral pain syndrome, have recently been validated, in which the hypersensitivity to distension results from the mastocytosis induced by intestinal parasitism (18), anaphylactic shock, or septic shock (unpublished observations). Measurements can either be limited to the determination of the threshold of reaction (first abdominal cramp) or take into account the intensity of the responses (number of abdominal cramps, number of identified postures, amplitude of the cardiovascular reflex response).

SPECIFIC MEDIATORS OF GUT HYPERSENSITIVITY

So far, no specific mediator has been identified that discriminates between visceral pain and somatic pain, although some mediators are more implicated in visceral hyperalgesic states. No mediator appears to be highly selective for either low- or high-threshold subpopulations of mechanical sensory neurons or neuronal populations encoding for immediate and late activation at the spinal cord level, except for \( \kappa \)-opioid receptors (21). Serotonin (5-HT) appears relatively selective of visceral nociception. This mediator is released at the intestinal level by enterochromaffin cells, platelets, and mast cell degranulation and subsequently acts on visceral afferents through specific receptors (for review, see Ref. 27). 5-HT is involved in the activation of primary afferents; studies on pseudoaffective (cardiovascular) reflex responses to gut distension have suggested an action through a 5-HT\(_3\) receptor subtype coupled to a sodium channel present on primary afferent endings. Indeed, 5-HT\(_3\) antagonists injected intravenously at low doses exert potent visceral analgesic effects in response to gut distension in different rat models of visceral pain; several studies, however, pointed out the heterogeneity of the responses to these antagonists in animal models of distension and the lack of dose-response relationships. Moreover, 5-HT\(_3\) receptor antagonists do not appear to be more efficacious in conditions of hyperalgesia (20). 5-HT\(_3\) receptors are also expressed within the central nervous system in limbic structures, brain stem, and spinal cord. At the dorsal horn level, they are localized presynaptically in the superficial laminae but also on intrinsic neurons. These receptors are expressed by enkephalinergic neurons and play a role in spinal analgesia produced by 5-HT (32). Some other 5-HT receptor subtypes, such as 5-HT\(_{1A}\), are involved at central and peripheral levels in the mediation of visceral nociceptive inputs. 5-HT\(_{1A}\) receptor agonists, such as 8-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), exert centrally-mediated antinociception on gastric distension (26), whereas 5-HT\(_{1A}\) antagonists have an antinociceptive effect at the rectocolonic level in hyperalgesia models (4). The
involvement of 5-HT₄ receptors in the modulation of visceral afferents is not known; however, 5-HT₄ receptor antagonists are able to potentiate the inhibitory effects of 5-HT₃ antagonists on visceral pain (28).

Bradykinin (BK) participates in the mediation of the hyperalgesia caused by irritant substances in several animal models. Two BK receptor types have been identified. Although most of the demonstrated pathophysiological actions of BK are mediated through the B₂ receptor type, there is increasing evidence that B₁ receptors, which preferentially bind the BK metabolite [des-Arg⁹]-BK, are selectively upregulated during processes that follow some types of intestinal tissue injury. Recently, a link between B₁ receptor activation and inflammation-induced nociception has been identified, but its site of expression remains unclear, particularly for visceral pain, even though B₁ receptors are chronically expressed in viscera and B₁ receptor activation results in the release of hyperalgesic substances such as prostanooids (1). Endogenous NGF released from mast cells under various stimuli may increase the primary afferent sensitivity to BK (16). At the visceral level, antinoceptive effects of BK antagonists have already been shown with NPC-567, a nonselective B₁ and B₂ receptor antagonist that decreases pain induced by intraperitoneal administration of acetic acid and urate crystals. B₂ antagonists are also active on acute inflammation-induced rectal allodynia (for review, see Ref. 3), and both B₁ and B₂ antagonists attenuate or suppress postinfection-induced jejunal hyperalgesia (18).

Tachyklinins have an important role in the transmission of nociceptive messages from the gut. Many C-afferent fibers have “silent receptors” for neurokinins that can be sensitized by inflammatory processes in peripheral tissues. Increased expression of both NK₁ and NK₂ receptors, as well as SP and neurokinin A (NKA), have been described at spinal and peripheral levels in pain-associated gut inflammation. The recording of primary afferent discharges at the dorsal horn level in response to colonic distension has largely confirmed that the blockade of NK₁ or NK₂ receptors reduces neuronal activation. Recently, a more intense effect has been found for a selective NK₃ receptor blocker, suggesting that neurokinin B (NKB) and its NK₃ receptors located at the periphery play a role in inflammation-induced gut hyperalgesia (15). Interestingly, immunohistological studies have identified NK₃ receptors on intrinsic primary afferent neurons (IPANs) but not on extrinsic afferent neurons (17). Moreover, NKB is coexpressed in neurons that also contain preprotachykinin A, which synthesize SP and NKA at the myenteric and submucosal plexus levels. The signaling role of IPANs and their NK₃ receptor pelvic nerve terminals is probably important in colonic hyperalgesia. In agreement with such hypotheses is the observation that NK₃ antagonists are not active on the firing of pelvic afferents in response to distension of the urinary bladder, which is devoid of IPANs (15). Indeed, IPANs play a primary transducer role: distension could activate stretch-sensitive ion channels on IPANs where NK₃ receptors, in response to NKB, may facilitate the distension-induced release of a signaling molecule which in turn activates primary afferents. The intraperitoneal injection of acetic acid induces visceral pain and inhibits gastric emptying in rats. Tachykinin NK₁ receptor antagonists, such as RP-67580, are able to selectively suppress the peritoneogastric motor inhibitory reflex, and the NK₂ receptor antagonist SR-48968 selectively reduces abdominal cramps. In contrast, abdominal surgery-induced gastric ileus does not seem to be modified by NK₃ antagonists. All of these data suggest that nociceptive messages from the inflamed rat peritoneum involve NKA rather than SP as mediator, and/or, at least, tachykinin NK₂ receptors. For the lower gut, even in a situation of normalgia, the same selectivity of effects was observed for NK₁ (CP-96345, RP-67580) and NK₂ (SR-48968, MEN-10376) receptor antagonists: NK₁ antagonists reverse rectal distension-induced colonic inhibition without affecting the abdominal response, and NK₂ antagonists selectively reduce visceral pain as assessed by the number of abdominal contractions. NK₃ receptors at the periphery participate in both rectocolonic inhibitory reflex and nociception triggered by rectal distension. Finally, from the presently available data on visceral pain in animal models, it can be concluded that NK₁ receptor blockade prevents visceral hyperalgesia related to inflammation through an anti-inflammatory action but is inactive against an established hypersensitivity, whereas both NK₂ and NK₃ receptor blockade reduce visceral pain by acting both centrally and peripherally for NK₂ receptors and only at the periphery for NK₃ receptors.

Calcitonin gene-related peptide (CGRP) is present in most splanchnic afferents, and CGRP immunoreactivity almost disappears from the gut after either splanchnic nerve section or treatment with the sensory neurotoxin capsaicin. About 50% of CGRP immunoreactive afferent neurons also contain SP/NKA. Moreover, CGRP released at the spinal cord from central endings of primary afferents is important in the development of visceral hyperalgesia. Alternatively, peripherally released CGRP may modify sensory inputs, causing changes in blood flow, smooth muscle contractions, immune reaction, and/or mast cell degranulation. The intravenous administration of the CGRP₁ receptor antagonist human (h)-CGRP-(8–37) suppresses the abdominal cramps observed after the intraperitoneal administration of acetic acid in awake rats and blocks the inhibition of gastric emptying induced by peritonitis, increasing its usefulness in the prevention of both functional inhibitory reflexes and pain. CGRP is also involved in the mediation of pain produced by lower gut distension. Thus the CGRP antagonist h-CGRP-(8–37) reverses the sensitizing effects (allodynia) of acetic acid on abdominal response to colorectal distension after intracolonic administration of acetic acid (24).

NMDA receptors are believed to play only a small part in the nociceptive response evoked by acute stimulation of normal somatic tissues but would play a much larger part in the hyperalgesic response to peripheral
injury and inflammation. The role of NMDA receptors in the transmission of nociceptive messages from the gut induced by colorectal distension has been suspected for a long time (5), but recent studies suggest that other excitatory amino acids, such as quisqualic and kainic acids, may be involved in the mediation of visceral pain through \(\alpha\)-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) receptors at the spinal cord level (29). Moreover, recent studies also suggest that glutamate and NMDA receptors are involved at the peripheral level in the sensitization of primary afferent terminals during inflammation of the gut or urinary tract (22). NMDA receptors and glutamate have also been detected in intrinsic sensory neurons at the intestinal level. The predominant subtypes of NMDA receptors located in the gut are not known, but NMDA receptor antagonists acting more selectively on NR\(_1\)/NR\(_{2B}\) subunits have recently been shown to exert marked visceral antinociceptive effects (unpublished observations). Interestingly, NMDA receptor ion channel blockers, such as namentine and ketamine, or the glycine site antagonist MRZ 2/576 inhibit nociceptive reflexes evoked from the normal ureter, suggesting that, in contrast to somatic pain, NMDA receptors are involved in the processing of acute nociceptive inputs from noninflamed viscera (22). Moreover, classical non-selective NMDA receptor antagonists strongly inhibit the nociceptive response due to the increased sensitivity provoked by visceral inflammation (5). Therefore, we can speculate that NMDA antagonists at certain doses may act selectively on visceral hyperalgesia.

Ion channels located either on primary afferents or postsynaptically at the spinal cord level are interesting targets for the development of visceral antihyperalgesic drugs. Drugs binding to the \(\alpha\)2\(\delta\)-subunits of calcium channels, such as gabapentin and pregabalin, prevent the hypersensitivity to colorectal distension induced by septic shock, TNBS colitis, or stress (unpublished observations). They are active at a lower dose on the hyperalgesia component than on the basal response to a mechanical stimulus and possibly have a central site of action. Similarly, compounds that inactivate voltage-dependent sodium channels may prevent the in vivo glutamate release at the spinal cord, impairing the transmission of the nociceptive message. Moreover, molecules derived from trimebutine (JO-1614) that bind to dorsal root ganglion neuron sodium channels have been shown to reduce the inflammation-induced rectal hypersensitivity in rats (25).

Many reports pointed out that \(\mu\)- and \(\kappa\)-opioid agonists lessen the nociceptive response to either peritoneal administration of irritants or intestinal distension (for review, see Ref. 3). It was also shown that \(\kappa\)-agonists may act peripherally to prevent visceral pain and are more active in inflammatory conditions. \(\kappa\)-Agonists interact on sensory neurons of the periphery, with receptors coupled to multiple high voltage-activated (HVA) calcium channels by a pertussis toxin (PTX)-sensitive G protein pathway; inhibition of calcium channel function likely contributes, at least in part, to the peripheral analgesic action of \(\kappa\)-agonists in visceral nociception (30). Somatostatin (SST) and its SST\(_1\) and SST\(_2\) receptors have been identified within the spinal cord, with a localization implying that they can play a modulatory role in pain processing comparable to that of \(\alpha\)2- and \(\alpha\)2-adrenergic receptors (Fig. 2).

FROM PRECLINICAL TO CLINICAL STUDIES

Although many substances have been tested on models of gut distension-induced nociception, only a few of them were evaluated in multiple models of hyperalgesia to distension in rats (Table 1). Indeed, the evaluation in multiple models is a prerequisite for selection of active compounds for clinical trials in FBD. Tachykinin NK\(_2\) receptor antagonists have been found active in many of these models, including Nippostrongylus brasiliensis postinfection-, stress-, and TNBS-induced colitis, but have not been tested on lipopolysaccharide-induced rectal allodynia. Despite recent data suggesting that they modulate the activity of primary afferents, their site of action remains unknown in these models. In addition, their evaluation in humans has not been published so far. \(\kappa\)-Agonists, like fedotozine, are active in several preclinical models of gut hyperalgesia; fedotozine has also been shown to increase pain threshold in IBS patients when infused intravenously (7). 5-HT\(_3\) antagonists, such as cimétidine and alossetron, are active in several models of distension, with a limited improvement of efficacy in models of hyperalgesia, including intracolonic glycerol-induced abdominal cramps (2), an effect unrelated to their influence on colonic tone. In all of these models, the efficacy of 5-HT\(_3\) antagonists is not dose related. A 3-wk treatment of IBS patients with alossetron does not affect the threshold of pressure inducing the first sensation of pain; however, this threshold occurs at a larger volume of inflation of the bag, evidencing a relaxatory effect on the distal colon that could partly account for the improvement of

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Table 1. Drugs active on various models of rectal hyperalgesia to distension in rats

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symptoms (8). 5-HT₄ receptor agonists, like tegaserod, have been recently described as active on symptoms in IBS constipated patients with an improvement of pain scores. However, until now a possible role of 5-HT₄ in the modulation of gut sensitivity has never been investigated. 5-HT₁A receptor antagonists, such as WAY-100645, are active in various models of lower gut hypersensitivity (4), whereas 5-HT₁A agonists, including 8-OH-DPAT, increase the threshold of pain response to gastric distension. In humans, both 5-HT₁A agonists and 5-HT₁D antagonists affect the threshold of gastric distension-induced pain, but only 5-HT₁D antagonists, like sumatriptan, are found to be active on the symptoms of non-ulcer dyspepsia (31). B₁ and B₂ receptor antagonists have also been found to be active in models of inflammation- and hypermastocytosis-induced rectal allodynia; however, they have not been tested by the oral route and never for visceral pain in humans. B₂ receptor antagonists are of special interest since B₂ receptors are expressed in the gut only under inflammatory conditions. Gabapentin and pregabalin, two selective agonists of the α₂δ-subunits of calcium channels, are able to modulate glutamate release at the dorsal horn level in rodents and have been found to be active in visceral pain induced by septic shock, inflammation, colonic glycerol, and stress, at doses similar to those active in many models of somatic and neuropathic pain. Until now, they have not been tested on visceral pain in humans. Several groups of investigators have demonstrated that octreotide, a cyclic SST analog, is able to increase the threshold of sensitivity to colorectal barostatic distension in healthy subjects and to restore a normal level of sensitivity in IBS patients (for review, see Ref. 3). In the upper gut of healthy subjects, SST also reduces the symptoms of perception of fullness in response to gastric distension. Subcutaneously administered octreotide has been shown to relieve chronic refractory epigastric pain symptoms severe enough to provoke nutritional impairment over long periods of time (1–2 yr) with only minor side effects (12).

CONCLUSIONS

Even though the presence of gut hypersensitivity to distension has not been demonstrated in all patients with FBD, abdominal pain remains a major cause of patients consulting a gastroenterologist. Furthermore, barostat studies have shown that the majority of patients experiencing pain have an increased sensitivity of at least one part of the gut. In these patients, pharmacological agents that selectively target visceral hypersensitivity may be used successfully. The recent discovery of key neuropeptides and other mediators that can be involved at peripheral and central levels, and the characterization of their receptors, has led to the development of many preclinical models with which to evaluate their possible involvement in gut hyperalgesia. Drugs acting selectively on these receptors are now available, but their ability to improve symptoms in clinical situations remains to be determined for the most promising agents.

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