Effects of inflammatory mediators on gut sensitivity

Lionel Bueno PhD, Jean Fioramonti PhD

L Bueno, J Fioramonti. Effects of inflammatory mediators on gut sensitivity. Can J Gastroenterol 1999;13(Suppl A):42A-46A. Over the past decade, attention has been paid to the role of visceral sensitivity in the pathophysiology of functional bowel disorders, especially irritable bowel syndrome, and visceral hypersensitivity is the most widely accepted mechanism responsible for both motor alterations and abdominal pain. Inflammatory mediators sensitize primary afferents, especially C-fibre polymodal nociceptors, favouring the recruitment of silent nociceptors that give rise to secondary spinal sensitization. After local tissue injury, the release of chemical mediators such as potassium ions, ATP, bradykinin and prostaglandin E₂ directly activate nerve endings and indirectly trigger the release of algescic mediators such as histamine, 5-hydroxytryptamine and nerve growth factor from other cells, which, in turn, stimulate proximal afferent nerve endings and silent nociceptors. Among the intermediary structures activated by inflammatory mediators and susceptible to the release of proalgescic substances, mast cells and platelets play a crucial role; however, immunocytes such as macrophages and neutrophils or sympathetic nerve terminals are also candidates. Moreover, events likely to activate synthesis of mediators by mast cells, such as stress and septic shock, also trigger colonic hypersensitivity. Prolonged visceral hyperalgesia may also depend on spinal sensitization. A number of substances are candidates to play a role at the spinal cord level in mediating painful and nonpainful sensations. Among them, substance P, dynorphins and glutamate play a pivotal role in postsynaptic sensitization, particularly during and after gut inflammation. Finally, despite the complexity of the relationship between inflammatory mediators and gut hypersensitivity, numerous results strongly suggest that alteration neuroimmune communications at the gut level may trigger a series of events that give rise to chronic changes in visceral sensitivity.

Key Words: 5-Hydroxytryptamine, Gut sensitivity, Hyperalgesia, Inflammation, Irritable bowel syndrome, Tachykinins

Effets des médiateurs de l'inflammation sur la sensibilité intestinale

RÉSUMÉ : Au cours de la dernière décennie, on a consacré beaucoup d'attention au rôle de la sensibilité viscérale dans la physiopathologie des troubles intestinaux fonctionnels, surtout du syndrome du côlon irritable et c'est principalement à l'hypersensibilité viscérale comme mécanisme que l'on rend responsable des altérations motrices et de la douleur abdominale. Les médiateurs de l'inflammation sensibilisent les afférents primaires, particulièrement les nocicepteurs polymodaux de la fibre C favorisant le recrutement des nocicepteurs silencieux qui donnent lieu à une sensibilisation spinale secondaire. Après une atteinte tissulaire locale, la libération de médiateurs chimiques, comme les ions potassium, l'ATP, la bradykinine et la prostaglandine E₂ activent directement les terminaisons nerveuses et déclenchent indirectement la libération de médiateurs algésiques comme l'histamine, la 5-hydroxytryptamine et le facteur de croissance nerveux à partir d'autres cellules qui, en retour, stimulent les terminaisons nerveuses afférentes proximales et les nocicepteurs silencieux. Parmi les structures intermédiaires activées par les médiateurs de l'inflammation et susceptibles de libérer des substances pro-algésiques, les mastocytes et les plaquettes jouent un rôle crucial. Par contre, les immunocytes, comme les macrophages et les neutrophiles, ou les terminaisons nerveuses sympathiques, sont également des candidats. De plus, les phénomènes susceptibles d'activer la synthèse des médiateurs par les mastocytes, comme le stress et le choc septique, déclenchent également une hypersensibilité du côlon. L'hyperalgésie viscérale prolongée peut également dépendre d'une sensibilisation spinale. Un certain nombre de substances pourraient jouer un rôle au niveau de la moelle épinière et amener les sensations douloureuses et non douloureuses. Parmi elles, la substance P, les dynorphines et le glutamate jouent un rôle central dans la sensibilisation post-synaptique, particulièrement durant et après les inflammations intestinales. En dernier lieu, malgré la complexité des rapports entre les médiateurs de l'inflammation et l'hypersensibilité intestinale, selon de nombreux résultats, une altération des communications neuro-immunitaires au niveau intestinal pourrait déclencher une série de phénomènes qui donnent lieu à des changements chroniques de la sensibilité viscérale.

Department of Pharmacology, INRA, Toulouse, France

Correspondence and reprints: Dr L Bueno, Department of Pharmacology, INRA, 180 Chemin de Tournefeuille, BP3, 31931 Toulouse Cedex 9, France. Telephone 33-561-28-51-41, fax 33-561-28-53-97, e-mail lbueno@toulouse.inra.fr

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Abdominal pain is the most frequent complaint of patients with functional bowel disorders and, in contrast to acute abdominal symptoms, cannot be relieved by a specific treatment of the disease. Visceral hypersensitivity is the most widely accepted mechanism responsible for both motor alterations and abdominal pain in functional bowel disorders. Indeed, the function of the gut is based on numerous intestino-intestinal motor and/or secretory reflexes. Alterations of these reflexes may initiate abnormal intestinal motor profiles, which can, in turn, generate pain.

Hyperalgesia of the gut may result from sensitization of primary afferent nerve endings, enhanced transmission of nociceptive inputs at the spinal cord level, alterations in the integrative processes of nociceptive messages to the cortex or defects in the activation of descending antinociceptive pathways. Even though the nature of hypersensitivity in

functional bowel disorders is not well known, there is increasing evidence that alterations in local neuroimmune interactions in the gut act as a buffer.

ALTERATIONS IN PRIMARY AFFERENT FUNCTION

Alterations in primary afferent function may correspond to a peripheral sensitization of primary afferents, especially C-fibre polymodal nociceptors, changes in endogenous pain-modulating systems and/or the recruitment of silent nociceptors (1).

After local tissue injury, a release of chemical mediators, such as potassium, H^+ -ATP and bradykinin, as well as inflammatory mediators (eg, prostaglandin E_2 [PGE_2]) can directly activate nerve endings (2,3). These substances trigger the release of algescic mediators such as histamine, 5-hydroxy-

Figure 1) Several structures activated during local inflammatory reaction may release inflammatory mediators to cause hyperalgesia. Macrophages are probably the most important. Several mediators such as 5-hydroxytryptamine (5-HT), adenosine (ADO), bradykinin (BK) and ATP are released in the medium from cell lyse (ATP, platelets, 5-HT, ADO) and vessels (BK). They play a direct action on nociceptors, but hyperalgesia may be due to substances, such as prostaglandin (PG) E_2 , histamine (HIST) and nerve growth factor (NGF), increasing either the permeability of an ion channel or producing a G protein-mediated increase in the activity of enzymes, leading in turn to an increase in cation permeability and a decrease in threshold. AA Arachidonic acid; CGRP Calcitonin gene-related peptide; G G protein; IL Interleukin; LTB₄ Leukotriene B₄; NA Noradrenaline; NPY Neuropeptide; SP Substance P; PIP₂ Phosphatidylinositol biphosphate; PMN Polymorphonuclear; TNF Tumour necrosis factor. Reproduced with permission from reference 25

TABLE 1
Substances having pronociceptive properties by acting peripherally

Agents acting directly on primary afferent	Agents acting indirectly via other cell types
Prostaglandin E ₂	Noradrenaline
Prostaglandin I ₂	IL-1, IL-8
8(R), 15S-diHETE	IL-6, TNF- α
Adenosine, ATP	NGF, NGF octapeptide
Bradykinin	Bradykinin
Serotonin (5-HT)	Leukotriene B ₄
Protons (low pH)	fMLP, C5a
Calcitonin gene-related peptide	Substance P, VIP
Substance P	

C5a Complement 5a; diHETE Dihydroxyeicosatetraenoic acid; 5-HT 5-Hydroxytryptamine; IL Interleukin; NGF Nerve growth factor; TMLP Formyl-methylnleucyl-phenylalanine; TNF Tumour necrosis factor; VIP Vasoactive intestinal polypeptide. Data from reference 24

tryptamine (5-HT), nerve growth factor (NGF), prostanoids from other cells and afferent nerves. They can, in turn, sensitize endings of afferent nerve terminals, resulting in an increased response to painful stimuli. Prostaglandins and other arachidonic acid derivatives increase the sensitivity of nerve terminals to bradykinin or other pain-producing substances, followed by a cascade of events leading to a secondary sensitization of nearby nociceptors, in which neuromediators such as substance P, histamine, 5-HT and cytokines play a role. In addition, the close proximity between mast cells and endings of sensory neurons results in an amplifying loop in which substance P released from nerve endings activates mast cell degranulation, releasing histamine, which further induces a release of substance P from sensory endings and NGF, which influences the development and function of sensory neurons (3).

Although mast cells appear to have a major role in the sensitization of primary afferents, there is increasing evidence that substances released from other local entities are important (Figure 1). With inflammation, prostaglandins released from both macrophages and sympathetic terminals may act directly on receptors located on afferent endings. ATP is released as a cotransmitter with noradrenaline from sympathetic nerves innervating visceral smooth muscle and can act at the P2 X₃ subtype purinoreceptor located on terminal endings to generate pain signals (4). Eicosanoids such as 8R, 15S di-HETE released from neutrophils may directly affect the adenylcyclase activity in afferent neurons. Moreover, cytokines secreted by macrophages may also indirectly affect neuronal activity (2).

Consequently, primary afferents may be permanently sensitized by increased amounts of algescic agents such as bradykinin, serotonin, eicosanoids or ATP acting directly on specific receptors located on neuron terminals or through substances such as interleukins that affect their release by immunocytes (Figure 1). These increased levels are characteristic of inflamed tissues and are thought to be responsible for persistent pain triggered by local inflammation because

acute administrations on these substances trigger a transient algescic state in animals.

NERVE PLASTICITY AND PERSISTENT REMODELLING

Nerve remodelling occurs during inflammation, which can trigger chronic hypersensitivity in the submucosa and other structures in the gut (5). These changes are complex, time-dependent and related to the nature of inflammation. For example, the acute phase of *Nippostrongylus brasiliensis* infection is associated with a 2.5-fold increase in the nerve content of the tissues, chiefly as a result of axonal dilation. However, during the recovery phase, when mast cell hyperplasia persists (14 to 28 days later), the mean cross-sectional areas of nerves decrease, while the diameter of small fibres increases. This is consistent with nerve regeneration, which can change sensitivity. Furthermore, recent data obtained in rats suggest that mucosal nerves are in a constant state of modelling, particularly B-50 immunoreactive nerves (6). Although it has been known for a long time that injured afferent fibres in damaged tissues become more sensitive to mechanical, chemical and probably thermal stimuli (7), more information is now available concerning the nature of the mediators involved, particularly mediators related to polymodal nociceptors. A direct increase in sensitivity at the level of the sensory neuron itself, presumably at its peripheral endings, is not the only way by which mediators enhance sensitivity. Thus, enhanced sensitivity may result from direct activation of a receptor opening calcium or sodium ion channels, or from up- or down-regulation of receptors on nerve endings associated with changes in the number and proximity of resident immunocytes, and the size and new distribution of sensory neuronal endings (2).

SENSITIZATION AT THE DORSAL HORN LEVEL

Peripheral injury of primary afferent sensory neurons, as well as the permanent activation from locally released direct and indirect algescic mediators, is associated with an increase in the excitability of the dorsal horn. This hyperexcitability state is important because the ability to amplify nociceptive inputs (also called the 'wind up' phenomenon) is still present hours to days after the peripheral irritating stimulus is no longer present. This hyperexcitability may play a role in the pathogenesis of hyperalgesia (7,8). It has also been postulated that an injury evokes action potentials that are conducted antidromically along branches of the nociceptor's axons or other terminal endings, where they cause release of algescic (pain-provoking) chemical substances that sensitize other nociceptors in this area. However, until now, no evidence of cross-sensitization between visceral and somatic afferents has been demonstrated, although they can project spinally on the same interneurons (8). The only evidence that visceral inflammation affects sensory inputs from the inflamed viscus as well as from skin-originating noxious stimuli was observed in a cat model of urinary bladder inflammation. Sensitization occurred at the level of the spinal cord, selectively increasing postsynaptic ascending messages (9). How-

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ever, it has been shown that only 40% of patients with Crohn's disease exhibit rectal hypersensitivity, suggesting that the 'wind up' phenomenon is not constant for visceral pain or is counterbalanced by activation of descending bulbospinal inhibitory pathways, as suggested by frequent associated somatic hypoalgesia (10).

MEDIATORS INVOLVED

A number of candidate mediators are released by local structures during inflammation and have a proalgesic effect when administered systemically in animals (Table 1). They act directly on primary afferents or indirectly on other cell types to induce the release of substances acting directly on afferent nerves. Several recent studies have identified the presence of receptors for serotonin, bradykinin, adenosine, ATP and PGE₂ on the endings of primary afferents.

Serotonin (5-HT) is involved in the activation of primary afferents, and studies of pseudoaffective (cardiovascular reflex) response to gut distension have suggested an action through a 5-HT₃ receptor subtype coupled to a sodium ion channel on primary afferent endings. Indeed, 5-HT₃ antagonists injected intravenously at low doses have potent visceral analgesic activity in response to duodenal distension rats (11); numerous studies using different animal models of visceral pain have confirmed such results. It is probable that some other receptor subtypes to 5-HT, such as 5-HT_{1A} are involved at the peripheral level in the mediation of visceral nociceptive inputs (12,13).

Bradykinin plays a major role in various inflammatory processes (14) and is involved in the mediation of pain and hyperalgesia caused by irritant substances in many animal models. Bradykinin receptors are subdivided into two categories, and most of the demonstrated physiological and pathophysiological actions of kinins are mediated by the B₂ type of bradykinin receptors, where bradykinin binds as the agonist. However, increasing evidence is accumulating that the B₁ receptors, which bind the bradykinin metabolite des-Arg⁹-BK with higher affinity, become more important in processes following some types of tissue injury, during which they are selectively upregulated. Bradykinin receptors are localized on nociceptive sensory neurons. At the visceral level, antinociceptive effects of bradykinin 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 (15). Suppression of carrageenan-induced hyperalgesia and hyperthermia by local administration of NPC-567 reveals the involvement of bradykinin in neuronal sensitization due to carrageenan (16).

Adenosine is a neuromediator in many nerve structures, and receptors of the A₂ type coupled to sodium ion channels are present on terminal endings of visceral primary afferents; however, its role in triggering nociceptive messages from visceral endings remains to be explored. Tachykinins and calcitonin gene-related peptide (CGRP) have an important role in the transmission of nociceptive messages from the gut. Many C-afferent fibres have 'silent receptors' for

neurokinins that can be sensitized by inflammatory processes in peripheral tissues. The involvement of substance P and its receptors in pain transmission is supported by a large body of experimental findings.

Increased expression of both natural killer (NK)₁ and NK₂ receptors, as well as substance P at the spinal cord level in hyperalgesic states associated with experimental arthritis, suggests that NK antagonists may also act at the central nervous system level to alter nociceptive messages to the brain. The NK₁ peptide antagonist GR 82334 administered intracerebroventricularly or intrathecally in mice exerts antinociceptive effects, especially towards chemogenic pain (17).

In rats, intraperitoneal injection of acetic acid induces visceral pain and inhibits gastric emptying. Capsaicin-sensitive C-fibres are involved in triggering both visceral pain and inhibition of gastric emptying. Recently, it has been shown that tachykinin antagonists are able to attenuate these responses with a selective action of NK₁ antagonist such as RP 67580 on the peritoneogastric motor inhibitory reflex, while the NK₂ antagonist SR 48968 selectively reduces abdominal cramps (18). In contrast to these results related to the acetic-induced peritonitis model, abdominal surgery-induced gastric ileus does not seem to be modified by an NK₁ antagonist. These results suggest that nociceptive messages from the inflamed peritoneum involve NK_A rather than substance P as mediator and/or at least NK₂ receptors in rats.

During graded rectal distension in the noninflamed rectum, systemic infusion of selective NK₁ (GR 73632) or NK₂ (GR 64349) receptor agonists have different effects. The NK₁ agonist enhances selectively the rectocolonic inhibitory reflex, while the NK₂ agonist increases selectively the number of abdominal cramps (19). The same selectivity of effects was observed for NK₁ (CP 96345, RP 67580) and NK₂ (SR 48968, MEN 10376) receptor antagonists. While NK₁ antagonists reverse rectal distension-induced colonic inhibition without affecting abdominal response, NK₂ antagonists selectively reduce visceral pain characterized by the number of abdominal contractions.

Finally, the involvement of substance P in visceral hyperalgesia related to gut inflammation was shown in chemically induced colitis in rats; a positive correlation was shown between increased concentrations of substance P and colonic inflammation with abdominal pain (20).

CGRP is present in a large number of splanchnic afferents, and CGRP immunoreactivity largely disappears from the gut after splanchnic section or treatment with the sensory neurotoxin capsaicin (21). About 50% of CGRP immunoreactive afferent neurons also contain substance P or NK_A immunoreactivity, and CGRP released from peripheral terminals of primary afferents is important in the development of visceral hyperalgesia. Alternatively, peripherally released peptides may modify sensory inputs by mediating axon reflexes, causing changes in blood flow, smooth muscle contractions, immune reactivity and/or mast cell degranulation.

When injected intracerebroventricularly in mice, CGRP inhibits the nociceptive response in the writhing test (22); however, in rats, intravenous administration of the CGRP₁ receptor antagonist (h-CGRP₈₋₃₇) suppresses the abdominal cramps observed after intraperitoneal administration of acetic acid in awake rats (23). Furthermore, in contrast to the selective NK₂ antagonists, this CGRP antagonist blocks the inhibition of gastric emptying induced by peritonitis.

Injected intravenously, CGRP mimics the influence of intraperitoneal acetic acid on both gastric and abdominal responses. Recently, it has been demonstrated that CGRP is involved in the mediation of pain related to lower gut distension. Indeed, the CGRP antagonist h-CGRP₈₋₃₇ was shown to reverse the sensitizing effects (allodynia) of acetic acid on nociceptive response to colorectal distension after intracolonic administration of acetic acid (23).

CONCLUSIONS

In humans as well as in experimental models, gut inflammation generates acute and/or chronic pain related to the type of inflammation. Substances synthesized by injured tissues as well as activated immunocytes may act directly on endings of nociceptive fibres to trigger nociceptive inputs to the spinal cord (prostaglandins, ATP, bradykinins) or may activate secondary structures producing a more prolonged release of sensitizing agents such as kinins, NGF, 5-HT and histamine. These immediate and prolonged stimulations of primary afferent endings, in turn, initiate a sensitization at the dorsal horn level associated with changes in receptor density and expression of neurotransmitters such as *N*-methyl-D-aspartate, resulting in chronic facilitation of nociceptive transmission from the gut, which must be considered in the etiology of functional bowel disorders.

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