New Concepts in Irritable Bowel Syndrome – Visceral Hypersensitivity, Inflammation and New Therapeutic Targets

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ABSTRACT

Irritable bowel syndrome is characterised by abdominal pain associated with alteration of bowel form or frequency, bloating, urgency, feeling of incomplete evacuation, straining and at times passage of mucus. Pain often brings patients to seek medical intervention, but currently, therapeutic options for targeting pain in this group of patients remain small, with most drugs giving disappointing results, or unacceptable side effects. Opiates have several side effects including respiratory depression, constipation, sedation and nausea. Tolerance often develops, and care needs to be taken during prescribing to avoid synergy with other drugs. Scientific advances have allowed a greater understanding of the mechanisms of pain. Animal models, such as the rat visceral hypersensitivity model and knockout animals, provide a tool for further advances and trial of new therapies. Understanding the mechanisms underlying the plasticity of the nervous system provides further insight into pain pathways. Learning more about neurotransmitters, receptors and nociceptor transduction, will allow targeted novel therapies to be formulated which should be more effective and have less unwanted effects. Focus on channels expressed solely by nociceptors, for example TRPV1 and P2X3, offers promise. Several other putative targets include NK, NGF, and Nav1.8 antagonists. At least in some instances, such visceral hypersensitivity may be triggered by infective episodes.

INTRODUCTION

Visceral hypersensitivity, defined by reduced pain and discomfort thresholds, is common in irritable bowel syndrome,[1,2] and leads to the symptoms of abdominal pain and perhaps urgency, bloating and feeling of incomplete evacuation. Although visceral hypersensitivity is not fully understood, several mechanisms have been proposed including psychosocial factors and altered sensorimotor function of the gut, a major component of which is believed to be due to peripheral and central sensitisation of visceral afferents. Further knowledge of receptors and neurotransmitters involved in visceral pain can provide a foundation for new pharmacological development.

VISCERAL HYPERSENSITIVITY AND INFLAMMATION

The pathogenesis of irritable bowel syndrome (IBS) requires consideration of visceral hypersensitivity, altered motility and psychological factors. The concept that irritable bowel syndrome (IBS) may follow infection was first hypothesised by Chaudhary and Truelove,[3] when 34 out of 130 IBS cases related their symptom onset to a bout of gastroenteritis. This has since been supported by further studies.[4-6] The relative risk of developing post-infectious IBS is 10-12 compared with uninfected controls. Persistence of inflammatory cells in colonic biopsies of such patients,[5,6] and an increase in mast cells seen in the colon and terminal ileum with increased concentrations of vasoactive intestinal peptide (VIP), and substance P (SP),[7,8] suggest that inflammatory changes are responsible for colonic hypersensitivity. Various animal studies have also shown an association between inflammation and visceral hypersensitivity, such as *Trichinella spiralis* infection in certain mice strains,[9,10] and gastrointestinal inflammation in mice and rats leading to hypersensitivity to distension.[11-13] Al-Chaer *et al*[14] carried out experiments in rats which revealed that colonic irritation in neonates led to an adult model of chronic visceral hypersensitivity. Similarly, IBS-type symptoms seen in quiescent ulcerative colitis,[15-17] is thought to be secondary to inflammation.

Painful stimuli activate peripheral visceral nociceptors which in turn signal receptor-ion channels and generate an action potential. Sensory afferent neurones express a large number of ion channels, receptors, neurotransmitters and neuromodulaters. Inflammation leads to enhanced nociceptive sensitivity, with an increased pain response to stimuli (hyperalgesia), or the sensation of pain to a normally non-noxious stimulus (allodynia).[18] Viscera receive dual innervation via vagal and spinal primary afferent neurones (Figure 1); the cell bodies of the vagal nerves lie in the nodose ganglia, and those of the spinal afferents in the dorsal root ganglia.[19] Visceral receptors can be classified into mechanoreceptors, and nociceptors. The afferent fibres are of three types; large myelinated, rapidly conducting A fibres which detect innocuous stimuli and small myelinated A fibres and unmyelinated polymodal C fibres both of which transmit

Table 1: Peripheral mediators and their receptors

Peripheral Stimulus/Ligand	Receptor	Mechanism of action
Capsaicin	TRPV1	Ion channel
Heat	TRPV1	Ion channel
	VRL-1	Ion channel
H^+	ASICs/DRASIC/TRPV1	Ion channel
ATP/adenosine	P2X	Ion channel
	P2Y	G-Protein coupled
PGE_2	EP	G-Protein coupled
Prostacyclin	IP	G-Protein coupled
Lipids	TRPV1	Ion channel
	PGE_2 / CB1	G-Protein coupled
Bradykinin, BK	BK_2	G-Protein coupled
Histamine	H1	G-Protein coupled
NGF	trkA	Tyrosine kinase
Serotonin	5-HT3	G-Protein coupled
Cannabinoids	CB1/CB2	G-Protein coupled
Substance P, SP	NK1	G-Protein coupled
Opioids	m d k	G-Protein coupled
Typtase (mast cells)	PARs	G-Protein coupled
Mechanical	??	??

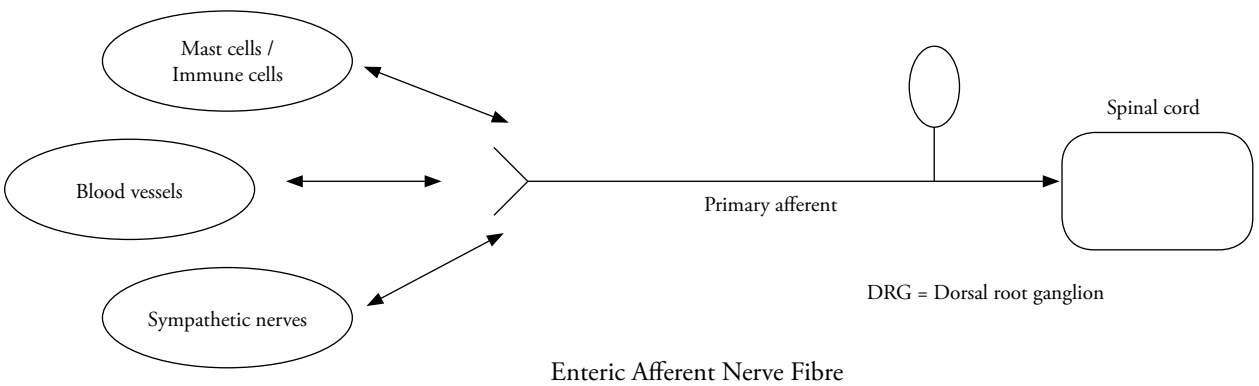


Fig. 1: Schematic diagram of enteric afferent nerve fibre and its connections.

noxious stimuli.[20] C-fibres are located in the muscle, serosa and mesentery, and A -fibres in the mucosa.[11] In hyperalgesia, both peripheral and central nervous system components are altered. Neuronal plasticity refers to the ability of sensory neurones to undergo changes in response to their environment, altering their transduction. This may be achieved by peripheral sensitization where the threshold of nociceptors is reduced, by central sensitization where dorsal horn neurone excitability is enhanced, or by altering the phenotype of sensory neurones. Nociceptors can be classified into high and low threshold receptors,[21] along with a group of so-called silent nociceptors which only respond following injury.[22] Altered signalling may be mediated via ligand- and voltage-gated neuronal ion channels, triggered by neurogenic inflammatory processes involving the release of mediators form sensory afferents,[23] recruitment of silent nociceptors,[24] nerve damage or immunological mechanisms. Inflammation itself can induce hypersensitivity through the release of inflammatory mediators including bradykinin, prostaglandins, protons, nerve growth factor (NGF) and serotonin, which originate in inflammatory mononuclear cells and entero-endocrine cells.[25] Nociceptors detect hot and cold, intense mechanical and chemical stimuli, including capsaicin, the pungent component of chilli peppers. Nociceptors include heat sensitive ion channels such as the vanilloid receptor VR1, the Na^+/ degenerin family such as ASIC (acid-sensing ionic channel) and DRASIC (dorsal root acid-sensing ionic channel), and receptors activated by chemical stimuli such as B2 receptors responding to bradykinin and EP receptors to prostaglandin. The amino acid glutamate is the main nociceptor neurotransmitter, which acts where the A - and C- fibres synapse onto the second order neurones located in the superficial laminae of the dorsal horn of the spinal cord. There are several types of glutamate receptors, including -amino-3-hydroxy-5 methyl-4-isoxazolepropionic (AMPA) receptors, N-methyl-D-aspartate (NMDA) receptors and metabotropic glutamate receptors (mGluRs).

PERIPHERAL INFLAMMATORY MEDIATORS AND CHEMICALS (Table 1)

A large number of chemicals can alter the sensitivity of visceral afferents. Tissue injury leads to release of several chemical mediators, including potassium ions, hydrogen ions, adenosine triphosphate (ATP) and bradykinin, along with inflammatory

mediators such as prostaglandin E_2, all of which directly activate nerve endings. Release of algesic mediators from other cells and afferent nerves, such as histamine, serotonin (5-HT), nerve growth factor (NGF), is promoted. As a result, afferent nerve terminals are sensitised and produce as increased response to pain.[26-28] Arachidonic acid derivatives, including prostaglandins, enhance the sensitivity of nerve terminals to bradykinin and other pain producing mediators. Substance P (SP), histamine, 5-HT and cytokines are involved in the sensitisation of proximal nociceptors. As mast cells lie in close proximity to sensory neurone endings, the release of SP from sensory nerve endings acts as a positive feedback loop resulting in mast cell degranulation and histamine release which in turn promotes further SP release, and NGF, which enhances the development and functioning of sensory neurones.[28] Mast cells also contain tumour necrosis factor- (TNF), interleukins, granulocyte-macrophage-colony stimulation factor (GM-CSF) and chemotactic agents.[29] SP contributes by inducing vasodilatation and plasma extravasation, as well as mast cell degranulation.[30] The importance of SP in mediating visceral hyperalgeisa has been illustrated by Cervero *et al*, who showed that in transgenic substance P receptor knockout mice, hyperalgesia did not develop following visceral inflammation as occurred with the control wild-type mice.[31] Histamine acts via histamine H_1 receptors to stimulate vagal and spinal afferents.[11,32]

Mast cell degranulation also releases the serine protease tryptase. Serine proteases act on protease-activated receptors (PARs), of which four have been identified. [33] PAR-1 and PAR-2 have been shown to be expressed by spinal afferents containing the transmitter calcitonin gene-related peptide (CGRP). PAR-2 receptors are activated by mast cell cell tryptase, and are G-protein coupled receptors. Activation of PARs leads to prolonged visceral hyperalgesia,[34] and PAR-2 stimulation in rat colon causes delayed hypersensitivity to colorectal distension.[35,36]

Bradykinin (BK), a nonapeptide of the kinin family, has been shown in humans to produce pain if applied to a cantharidin-induced blister base,[37] injected into the abdominal cavity,[38] or into the cephalic or brachial vein which has been sensitised with serotonin.[39] The effects of bradykinin are due to both activation especially of high threshold C-fibre associated nociceptors,[40] and sensitisation of nociceptors. Bradykinin has been shown to act via B_2 bradykinin receptors, with a specific B_2 receptor antagonist (HOE 140) shown to inhibit carrageenin-induced hyperalgesia in animal models.[41] Results of other animal experiments have suggested a role for both $B_1$and B_2 bradykinin receptors in hyperalgesia[42,43] involving induction of cytokines, including TNF and interleukin 1 .[44] A non-selective B_1/B_2 receptor antagonist, NPC-567, decreased pain induced by intraperitoneal acetic acid and urate crystal application in animals.[45] Prostaglandin E_2 (PGE_2) sensitises visceral nociceptors to BK via EP2 receptors during inflammation.[46] It is postulated that B_2 receptors are important in the initiating stages of inflammatory pain, and B_1 receptors in maintenance of persistent pain. B_2 receptors are found on sensory neurones,[45,47] unlike B_1 receptors which are located on other cells.[48]

ATP (adenosine triphosphate), which acts as a co-transmitter for noradrenaline, is released from local sympathetic nerves. ATP can act by activating G protein coupled receptors in nociceptor terminals via P2Y receptors,[49] or at the P2X purine receptors by activating ligand-gated receptors.[50]

Serotonin, released by enterochromaffin cells and platelets, activates primary afferents via 5-HT_3 receptor coupled to a sodium channel.[51,52] Low dose 5-HT_3 antagonist infusions in rats produced visceral analgesia in response to duodenal distension,[53] which has been reproduced in other studies. Pain has been shown to be promoted by 5-HT,[54] and not abolished fully by one selective 5-HT antagonist, suggesting the role of other 5-HT receptor subtypes in nociception, such as $5HT_{1A}$ receptors.[55,56] 5-HT_3 receptor antagonists such as alosetron have shown promise in alleviating pain in IBS.[57]

Prostanoids, including prostaglandins (PGs) and leukotrienes, are generated from arachidonic acid via cyclo-oxygenase and lipo-oxygenase enzymes, and play an important role in production of hyperalgesia. Prostaglandins, including PGE_2 and prostacyclin (PGI_2), are produced by a variety of cells, and act on EP_{1-4} and IP receptors located on primary afferents.[58,59] In rat jejunum, afferent fibres are stimulated by PGs initially via EP_1 receptors, with more prolonged sensitisation via EP_2 receptors.[60] PGE_1 and PGI_2 directly increase the activity of nociceptors, and PGE_2 has been shown to stimulate substance P release *in vitro*.[61,62] PGs act by reducing the activation threshold of sensory afferents,[61,63,64] or increasing membrane sodium conductance.

A_2 adenosine receptors coupled to sodium channels are found on visceral primary afferent terminals, but their role in nociception remains uncertain.[51] Adenosine has been found to cause pain on administration upon a skin blister.[65]

Tachykinins are peptides including substance P, neurokinin A (NKA), and NK(B), which act on NK_1, NK_2 and NK_3 respectively. In rat chemical-induced colitis a positive correlation was seen between increased concentrations of substance P and colonic inflammation with abdominal pain.[66] Studies, however, also point to a role for NKA at NK_2 receptors. In rat models, the pain induced by intraperitoneal injection of acetic acid, was reversed by the NK_2 receptor antagonist SR 48968.[67]

In experiments on non-inflamed rectum in rats, during graded rectal distension, the infusion of selective NK_1(GR 73632) or NK_2 receptor agonists, had differing effects. The NK_1 agonist enhanced the retrocolonic inhibitory reflex, whilst the NK_2 agonist resulted in an increased frequency of abdominal cramps.[68] The effects were reversed selectively by the appropriate receptor antagonists.

Entero-endocrine cells release cholecystokinin (CCK), which acts on vagal mucosal afferents.[69] CCK reduces opioid analgesia effects[70,71] by stimulating CCK-B receptors.[72] Infusing CCK in IBS patients has induced their usual pain,[73] supporting the theory of CCK having a pro-nociceptive effect.

Opiates are known to give effective relief in visceral pain. However, the effects may be mediated through opioid receptors located peripherally or centrally in the brain and spinal cord. Peripheral effects of opiates have been illustrated,[74,75] and , and opioid receptor antagonists alleviate pain induced by peripheral irritation at concentrations which are ineffective for systemic action. It is thought that opiates may act by inhibiting action potential conduction from sensory afferents,[76] or the

release of excitatory transmitters.[77,78] In anaesthetised rats, intracerebral or intrathecal administration of the morphine results in alleviation of pain induced by colorectal distension, as there is a central as well as peripheral mechanism of action.[79]

Cannabinoid CB_1 and CB_2 receptor agonists in animal models cause suppression of inflammatory hyperalgesia.[80,81] CB_1 receptors are mainly centrally located,[82,83] whereas CB_2 receptors are found on peripheral immune cells,[82,84,85] and not in the central nervous system (CNS).[82] CB_2 selective receptor agonists have been shown to be effective in inhibiting hyperalgesia, without CNS-mediated cannibinoid effects such as hypothermia.[86,87] They may therefore provide a potential effective agent in the use of visceral hypersensitivity and pain. Experimental use of selective CB_2-receptor agonists in animals, such as GW405833 systemically,[88] and AM1241 peripherally,[89,90] have shown promising results.

CONDUCTION OF NOCICEPTIVE SIGNALS-VOLTAGE-GATED SODIUM CHANNELS

Voltage-gated sodium channels (VGSC), of which there are ten functional types in the central and peripheral nervous system,[91] are responsible for the rising phase of the action potential[92] by a voltage-dependent increase in sodium ion permeability. They are involved along with potassium channels, in determining the excitability of sensory neurones, and consist of a transmembrane protein with a 260kDa subunit, and additional subunits. Two types exist- those sensitive to the potent puffer-fish toxin tetrodotoxin (tetrodotoxin-sensitive, TTXs), and the second group which are insensitive to tetrodotoxin (tetrodotoxin-resistant, TTXr).[93] TTXs channels are found in all sensory neurones, but TTXr channels are only located on nociceptor sensory afferents, and are slower to activate and inactivate.[92] TTXr sodium channels are likely to play an important role in nociceptive transmission to the CNS.[94] There is particular interest in the TTXr VSGC subunit SNS Nav1.8,[95] present in small diameter sensory neurones and dorsal root ganglia, which is activated by inflammation in the gut, and its conductance enhanced by PGE_2, adenosine and serotonin.[91] Experiments on Nav1.8 null mutant mice,[95] and animal models of colitis,[96,97] support a role for the Nav1.8 channel in visceral hyperalgesia and nociception. With respect to TTXr sodium channels, hyperalgesic mediators such as PGE_2, serotonin and adenosine, have been shown to increase the rates of activation and inactivation, decrease the activation threshold, and increase the size of the current.[98] These changes are likely to be mediated by phosphorylation of proteins, and PGE_2 has been shown to activate cAMP-dependent protein kinase A, PKA.[99]

NOCICEPTOR AFFERENT C-FIBRES

The polymodal small diameter C fibres of visceral afferents may be subdivided on the basis of histological markers into two main groups, with distinct trophic reqirements. One group, consisting of about 50% of C-fibres, contains the neuropeptides substance P and calcitonin-gene related peptide (CGRP), and is regulated by nerve growth factor (NGF). NGF is a molecule that promotes the survival and differentiation of sensory neurons and elicits many of its classical neurotrophic actions through its high affinity receptor trkA.[100] Increased trkA expression has been recently reported in inflammatory bowel disease.[101] CGRP is a neuropeptide found in both small and medium sensory neurons. In the gut, CGRP immunoreactivity is localised mainly to capsaicin-sensitive extrinsic afferent fibres but may also be found on a number of intrinsic neurons and processes. It is thought to play an important role in visceral afferent sensitisation.[102,103] Approximately half of the CRGP-immunoreactive neurones in rats also contain substance P.[104] SP is a putative excitatory neuro-neuronal transmitter, a vasodilator, as well as a mediator in inflammatory processes. Experimental colitis studies suggest that substance P is also involved in visceral hyperalgesic mechanisms. The other group of polymodal C fibres is identified by the presence of enzymes such as fluoride-resistant acid phosphatase or can be surface-labelled for isolectin B4 (IB4), and expresses $P2X_3$ ATP-gated ion channels. This second group of polymodal C fibres is dependent on glial cell line-derived neurotrophic factor (GDNF) for regulation of its physiological properties,[105] and lacks trkA receptors. Both groups of C fibres respond to similar types of stimulation including noxious thermal, mechanical and chemical stimuli, and the majority express the VR1 receptor.

NERVE GROWTH FACTOR (NGF)

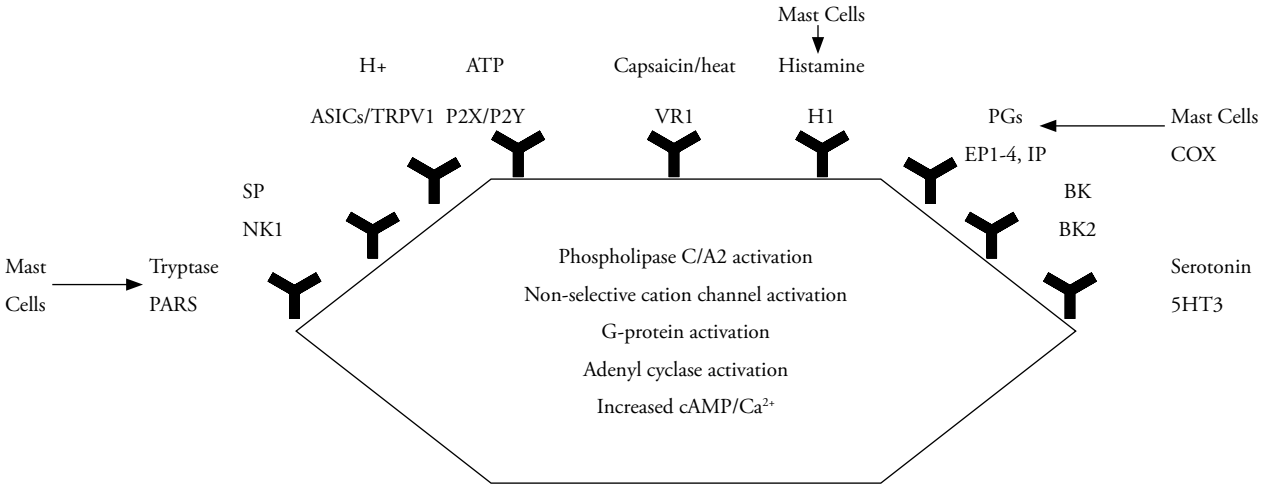
NGF has a major role in inflammatory hyperalgesia.[106,107] After binding to the high affinity receptor tyrosine kinase trkA, internalisation, and retrograde transportation to the cell body occurs. Here, survival, growth and phenotype of immature neurones is controlled via activation of second messenger systems,[108,109] including mitogen-activated protein(MAP) kinase signalling pathways.[110] NGF given systemically has been induce thermal and mechanical hyperalgesia in rats,[111] mice[112] and in humans.[113]

Levels of IL-1 , a cytokine produced by a variety of cells including monocytes, macrophages, fibroblasts, lymphocytes and smooth muscle cells, are increased during inflammation.[114,115] Experimental studies have revealed that administration of IL-1 induces hyperalgesia,[116,117] and NGF production by cells.[118,119] The hyperalgesic effects of IL-1 have been postulated to be mediated via prostaglandins,[116,120] via bradykinin B_1 receptor receptors,[121] or by nociceptor direct activation.[122]

NGF enhances levels of SP and CGRP in sensory neurones, and hence may contribute central sensitization in inflammatory states. TNF , a cytokine which is produced by inflammatory and other cells, is increased during inflammation. Local or systemic administration evokes hyperalgesia.[123,124] Anti-NGF reduces the hyperalgesic effects of both IL-1 ,[125] and TNF ,[126] suggesting these cytokines mediate their effects via NGF. NGF acts on cells expessing the trkA receptor, including inflammatory cells and small-diameter sensory neurones. Mast cells are an important target for NGF which promotes their growth, survival and degranulation.[127,128] Overall there is a potential for the development of new analgesic agents which antagonise NGF, IL-1 and TNF- to combat the pain of visceral hypersensitivity.

CENTRAL SENSITISATION - SENSITISATION OF DORSAL HORN NEURONES

An increase in the excitability of dorsal horn neurones, or the wind-up phenomenon, participates in the development of visceral hyperalgesia. Peripheral nociceptor inputs trigger the central enhanced responsiveness which persists after the initiating stimulus has subsided. Modulation is via triggering of intracellular signalling cascades which enhance post-synaptic transmission and



Some of the gastrointestinal nociceptor afferent mediators, their receptors and mechanisms of activation and sensitisation

Fig. 2 : Schematic diagram showing gastrointestinal nociceptive receptors and mediators.

depress inhibition, leading to an increased response to noxious and innocuous stimuli and hypersensitivity to regions extending beyond the inflamed area. Primary afferent C-fibres synapse onto second order neurones in specific superficial laminae in the dorsal horn, releasing the neurotransmitter glutamate which acts on fast responding AMPA, slower NMDA, and metabotropic mGlu receptors on post-synaptic spinal neurones. The synapses are inhibited by spinal inhibitory neurones with enkephalins and gamma-amino-butyric acid (GABA) as neurotransmitters. Other neurotransmitters such as substance P which acts on NK-1 receptors, CGRP, and neurotrophins including brain-derived neurotrophic factor (BDNF), are also released by primary afferent terminals and modulate the synaptic connection. Signals are then projected by neurones from the dorsal horn to the thalamus, brainstem and midbrain, and finally on to the cortex where pain is perceived.

Neuronal windup is a result of the suppression of Mg^{2+} blockade of the NMDA receptor, and occurs due to a cumulative depolarisation by summation of nociceptor-evoked synaptic potentials. Activation of the various receptors including the G protein coupled receptors such as NK1, EP and mGlu receptors, and tyrosine kinase receptors such as TrkB for BDNF, results in increased calcium influx and release of calcium from intracellular stores.[129] Calcium-dependent enzymes such as protein kinase C and calmodulin kinase, as well as protein kinase A via the G-protein coupled receptors, and tyrosine kinases via the TrkB receptor, are subsequently activated. The kinases phosphorylate NMDA and AMPA receptors, altering their function and inducing central sensitisation.

Central sensitisation also results from depression in signals from GABA-nergic spinal inhibitory neurones, due to a post-synaptic calcium increase and NMDA receptor activation. Finally, gene expression is altered in sensory neurones; genes encoding for nociceptor neurotransmitters, receptors and ion channels, are all upregulated,[130] probably by effects mediated via NGF.

PRIMARY SENSORY NEURONES AND ACTIVATION VIA DIFFERENT MODALITIES

Afferent nociceptors are activated by thermal, mechanical and chemical stimuli.

Heat

Noxious heat activates nociceptors at a threshold of approximately 40 degrees, via the VR1 (also known as TRPV1- transient receptor potential vanilloid receptor 1) cation selective channel.[131] This forms part of the transient receptor potential (TRP) family of ion channels, which are 6-transmembrane domain calcium channels.

Cold

CMR1 (cold/methanol receptor) or TRPM8,[132,133] a member of the TRP family, is a receptor which responds to cold, and is also activated by methanol.

Low pH

Protons act on the TRPV1 receptor, and other proton-gated ion channels including ASICs (acid sensing ion channel) and the ASIC homologue, DRASIC.

Mechanical

Mechanosensitive channels on sensory neurones include the epithelial sodium channel/degenerin family, and ATP-gated channels such as P2X and P2Y receptors.

ION CHANNELS INVOLVED IN NOCICEPTION (Fig. 2)

TRPV1/VR1 receptor

Caterina *et al* first identified the capsaicin receptor TRPV1, or VR1, in 1997 using an expression cloning strategy on cDNA from rat sensory neurones.[131] Capsaicin is the natural component of chilli peppers responsible for making them taste hot. Although nociceptors are partially characterised by their capsaicin sensitivity, prolonged exposure to capsaicin desensitises

nociceptor terminals, and explains its analgesic effects for which it is used in arthritis, post-herpetic neuralgia, and painful neuropathies. TRPV1 is expressed on small to medium-sized neurones throughout the nervous system, and on mononuclear blood cells.[134] The nucleotide sequence of TRPV1 predicts a protein of 838 amino acids with a molecular mass of 95,000. It consists of six transmembrane domains, with a hydrophobic loop located between the fifth and sixth.[131] TPRPV1 is activated by capsaicin and its analogues, lipids, other molecules such as resiniferatoxin that contain a vanillyl moiety, as well as by endocannabinoids including anandamide.[131,135] Upon activation, a sensation of burning pain is evoked, along with release of the neuropeptides substance P and CRGP. The vanilloid moiety is a common feature of capsaicin and resiniferatoxin. The action of TRPV1 is blocked by the competitive antagonist capsazepine, or the non-competitive antagonist ruthenium red. The receptor is also gated by noxious heat (>40 degrees), and its mechanism potentiated by protons. It has been suggested that inflammatory and ischaemic hyperalgesia may in part be mediated by the enhanced TRPV1 response resulting from a decreased tissue pH and production of excess hydrogen ions.[131,136]

The receptor is a nonselective cation channel with high calcium permeability; it is approximately ten times more permeable to Ca^{2+} than monovalent ions. Ca^{2+} excessive influx is likely to account for capsaicin-induced primary afferent nociceptor death which is seen on chronic exposure.[137] TRPV1 activity is subject to modulation by inflammatory mediators including bradykinin and PGs, probably by PKC or PKA mediated phosphorylation of the receptor.[138]

At least four other related proteins of the TRP family of ion channels have been identified since the discovery of TRPV1.[139] VRL-1 has similarities to TRPV1 in that it is also a non-selective cation channel with high Ca^{2+} permeability, and is heat-gated at a higher temperature of 52 degrees, suggesting a role in detection of noxious heat.[140] VRL-2 (or TRP-12) is an osmotically sensitive channel, which may respond to mechanical stimulation.[141] The epithelial Ca^{2+} channels (ECACs) ECAC1[142] and ECAC2[143] are highly Ca^{2+} permeable, and are activated by a reduction in Ca^{2+}, suggesting a role in calcium homeostasis.

TRPV1 has been shown to be upregulated both in patients with actively inflamed bowel,[144] and with rectal hypersensitivity.[145] The study by Chan *et al*[145] in rectal hypersensitivity revealed that the increase in nerve fibres expressing VR1 correlated with hypersensitivity to rectal distension and heat sensation. This suggests a role for TRPV1 in inflammatory-induced pain and visceral hypersensitivity. VR1 knockout mice have been shown to be almost completely insensitive to capsaicin, and demonstrate reduced inflammatory-induced thermal hyperalgesia.[146] VR1 antagonists therefore offer the potential as drugs to control pain in visceral hypersensitivity states, where VR1 is upregulated.

ATP-Gated ion channels:

Ion channels which are gated by extracellular ATP have been characterised on sensory neurones,[147,148] which respond to micromolar concentrations of ATP. Two types of receptor exist; P2X receptors are ATP-gated ion channels, and the G-protein coupled receptors activated by ATP are called P2Y.[149] Since the first P2X receptor was cloned in 1994,[150,151] seven further members, including P2X3, have been identified.[152] Sensory neurones express mRNA for six of the P2X receptors. P2X3 receptors are expressed on nociceptors,[153] and are involved in the ATP-gated current generated by them. Rat experiments revealed application of a P2X agonist induced paw pain, which was subsequently blocked by local anaesthetic,[154] suggesting a peripheral mechanism of action of nociception. Selective antagonists for P2X receptors may offer promise for therapeutic development in the future.

Acid-Sensing Ion Channels:

Tissue damage, whether due to inflammation, ischaemia, or infection, causes local acidosis and pain. This may be due to modulation of receptors, as in the case of the TRPV1 receptor, or direct activation. The first acid-sensing ion channel (ASIC) named ASIC1, a proton-gated cation channel expressed throughout the nervous system, was cloned in 1997.[155] The ASIC structure is comprised of two transmembrane domains, and it is a member of a family that includes the amiloride-sensitive epithelial Na^+ channel involved in renal sodium reabsorption, the mammalian degenerin channels (MDEG) responsible for neuronal cell death in nematodes, and the dorsal root acid-sensing ionic channel (DRASIC) expressed in dorsal root ganglion sensory neurones. ASIC1 is sodium selective channel on sensory neurones, and is closed at a pH of 7.4 but is activated once the pH falls below 7.0.[155] The related ASICs have been renamed as ASIC2a, ASIC2b, and ASIC3 (originally MDEG1, MDEG2, and DRASIC respectively),[156] and like ASIC1, all are sodium-selective, and amiloride-sensitive.

SUMMARY

Although IBS is likely to be multifactorial in origin, visceral hypersensitivity has been cited as a major factor in the pathophysiology, with support from experimental and clinical data in both animal models and humans. Visceral hypersensitivity is present in two-thirds of patients with irritable bowel.[157,158] Irritable bowel-like symptoms also occur in inflammatory bowel disease patients, with one-third of patients with ulcerative colitis and 42-57% of Crohn's disease patients in remission being affected,[159,160] probably secondary to inflammatory-induced visceral hypersensitivity which persists despite resolution of the acute attack. A common link may be inflammation resulting from previous infective gastroenteritis triggering visceral hypersensitivity via a number of key molecules and receptors discussed above. In others visceral hypersensitivity may be centrally-mediated.

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