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REVIEW

Enteric P2X receptors as potential targets for drug treatment of the irritable bowel syndrome

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The irritable bowel syndrome (IBS) is a gastrointestinal motility disorder affecting millions of patients. IBS symptoms include diarrhea, constipation and pain. The etiology of IBS is due partly to changes in the function of nerves supplying the gastrointestinal tract, immune system activation and to psychological factors. P2X receptors are multimeric ATP-gated cation channels expressed by neuronal and non-neuronal cells. Sensory nerve endings in the gastrointestinal tract express P2X receptors. ATP released from gastrointestinal cells activates P2X receptors on sensory nerve endings to stimulate motor reflexes and to transmit nociceptive signals. Antagonists acting at P2X receptors on sensory nerves could attenuate abdominal pain in IBS patients. Primary afferent neurons intrinsic to the gut, and enteric motor- and interneurons express P2X receptors. These neurons participate in motor reflexes. Agonists acting at enteric P2X receptors may enhance gastrointestinal propulsion and secretion, and these drugs could be useful for treating constipation-predominant IBS. Antagonists acting at enteric P2X receptors would decrease propulsion and secretion and they might be useful for treating diarrhea-predominant IBS. Current knowledge of P2X receptor distribution and function in the gut of laboratory animals provides a rational basis for further exploration of the therapeutic potential for drugs acting at P2X receptors in IBS patients. However, more information about P2X receptor distribution and function in the human gastrointestinal tract is needed. Data on the distribution and function of P2X receptors on gastrointestinal immune cells would also provide insights into the therapeutic potential of P2X receptor agents in IBS.
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Abbreviations: AH, afterhyperpolarization; ENS, enteric nervous system; GI, gastrointestinal; IBS, irritable bowel syndrome; I_K, intermediate-conductance calcium-activated potassium current; PPADS, pyridoxal phosphate 6-azophenyl-2′, 4′-disulfonic acid

Introduction

The irritable bowel syndrome (IBS) is a functional bowel disorder that is associated with altered gastrointestinal (GI) motility and changes in defecation. Abdominal discomfort and pain particularly during defecation are also characteristic symptoms of IBS (Drossman *et al*., 2002). While it is uncertain if there are changes in the function of enteric nerves controlling GI motility in IBS patients, it is clear that there are changes in the function of the sensory innervation of the GI tract. Visceral hypersensitivity or lower pain thresholds for affective responses to GI distention is a common symptom in IBS patients and this is likely to contribute to the abdominal pain that occurs during defecation and contractions of the GI muscle layers (Mayer, 2000; Drossman *et al*., 2002). Although visceral hypersensitivity has been associated with decreased pain thresholds in IBS patients, decreased thresholds for activation of sensory nerves could also contribute to GI motor hyperreflexia leading to defecation urgency and diarrhea.

The pathophysiology of IBS is complex and incompletely understood. There are disturbances in normal GI motility patterns in many but not all IBS patients (Drossman *et al*., 2002) and, therefore, the neurohumoral mechanisms producing normal GI motility may be altered. As mentioned above, the function of sensory nerves supplying the GI tract is altered, and central nervous system mechanisms responsible for processing visceral input and pain sensations are different in IBS patients compared to control subjects (Mayer, 2000). Psychological and cognitive factors also play a prominent role in the etiology of IBS (Drossman *et al*., 2002; Talley, 2003). Finally, interactions between the immune system and neurohumoral control of GI motility may play a role in the pathophysiology of IBS. This last conclusion is based on reports of many IBS patients who indicate that the onset of GI symptoms occurred subsequent to an acute episode of gastroenteritis (Spiller, 2003). It is feasible that immune activation in the GI tract may produce long-lasting changes in the function of neuronal and non-neuronal cells in the gut. For example, mast cell signaling molecules such as histamine and tryptase (Wood, 1992; Liu *et al*., 2003; Reed *et al*., 2003) as well as cytokines and other inflammatory mediators (Frieling *et al*., 1997; Xia *et al*., 1999; Kelles *et al*., 2000; Liu *et al*., 2003; Neunlist *et al*., 2003) cause acute changes in excitability of enteric neurons. Intestinal inflammation is also associated with long-lasting changes in the expression of a

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variety of proteins that regulate cell excitability, growth or differentiation of enteric neurons (Poli *et al*., 2001; Sharkey & Kroese, 2001). Primary afferent neurons supplying the GI tract are also targets for the actions of inflammatory mediators and cytokines (Furness & Clerc, 2000; Holzer, 2001; Moore *et al*., 2002; Stewart *et al*., 2003). Inflammatory mediators can also change the function and number of glial cells in the enteric nervous system (ENS) (Bradley *et al*., 1997; Ruhl *et al*., 2001; Sharkey & Kroese, 2001). Inflammation-induced changes in the function of enteric and primary afferent neurons and enteric glia would all contribute to the spectrum of GI symptoms characteristic of IBS.

Given the complex pathophysiology of IBS, it is not surprising that it has been a challenge to develop drugs that effectively and safely treat the symptoms of IBS in all patients (Kamm, 2002; Talley, 2003). Another obstacle to the development of new drugs for the treatment of IBS has been the shortage of appropriate animal models of IBS against which potential medications can be screened (Mayer & Collins, 2002). In addition, IBS is not a homogenous disorder, as three broad classes of IBS patients have been defined using clinical criteria. The first group includes patients with diarrhea as the predominant symptom (diarrhea-predominant IBS). The second group of patients exhibits constipation as their predominant symptom (constipation-predominant IBS), while the third group of patients has alternating symptoms (alternating-predominant). Therefore, a single drug or drug class is unlikely to be effective in the treatment of all IBS patients (Corazziari *et al*., 2003). Another issue relevant to drug development is that psychological and cognitive factors are prominent in IBS and there is a strong placebo response in all trials of potential IBS medications (Talley, 2003). Therefore, differences between placebo and treatment groups in efficacy outcomes are often small (Corazziari *et al*., 2003; Kamm, 2002; Talley, 2003). Despite these challenges, there has been some success in developing drugs for treatment of the symptoms in IBS patients. Alosetron, an antagonist at 5-HT_3 receptors, reduces abdominal pain and stool frequency in some female, diarrhea-predominant IBS patients (reviewed by Lembo *et al*., 2003; Mayer & Bradesi, 2003). Although, alosetron has a demonstrated therapeutic benefit in this subset of IBS patients, alosetron therapy has been associated with some occurrences of severe constipation and ischemic colitis (Lembo *et al*., 2003; Mayer & Bradesi, 2003). These adverse events resulted in a short-term withdrawal of alosteron from the market and subsequent re-introduction with marked restrictions on its use. However, more recent data indicate that the occurrence of ischemic colitis or other adverse events in alosetron-treated IBS patients may not be greater than that in alosetron-untreated IBS patients (Miller *et al*., 2003). Tegaserod, a 5-HT_4 receptor partial agonist, has been introduced recently for the treatment of female constipation-predominant IBS patients (Wagstaff *et al*., 2003). Tegaserod increases stool frequency and reduces abdominal bloating and pain (Novick *et al*., 2002), and up to this time no significant adverse effects have been reported to occur during tegaserod treatment.

Despite encouraging developments in drug therapies, the prevalence of IBS and heavy health-care burden posed by this disorder have led to a continued search for new classes of drugs for the treatment of IBS (Callahan, 2002, see other articles in this supplement). In the search for new drugs, two important considerations will impact on the types of drugs likely to be effective in treating IBS. As there are changes in motility and in the function of the sensory innervation of the gut, a drug candidate would need to target both the sensory nerves involved in visceral sensation and the neurohumoral mechanisms controlling GI motility. P2X receptors for ATP are expressed by both extrinsic intestinal sensory nerves and by enteric nerves controlling GI motor reflexes (Kirkup *et al*., 1999; Holzer, 2001; Galligan *et al*., 2000; Kennedy *et al*., 2003). In addition, immune cells express P2X receptors (DiVirgilio *et al*., 2001), and agonists acting at these receptors cause immune activation and release of cytokines and other signaling molecules that can change sensory and enteric nerve function (Sharkey & Kroese, 2001; Sharkey & Mawe, 2002). Therefore, drugs acting at P2X receptors might be useful agents for the treatment of sensory and motor abnormalities in IBS. This review will discuss the role of P2X receptors on nerves supplying the GI tract in order to provide a rationale basis for the development of P2X receptor ligands for the treatment of IBS.

The enteric nervous system

The ENS is the division of the autonomic nervous system whose nerve cell bodies and non-neuronal support cells reside entirely within the gut wall (Furness & Costa, 1987). The ENS is composed of two nerve plexuses: the myenteric plexus and submucosal plexus. The principal function of the myenteric plexus is to control GI motility, while the submucosal plexus controls the secretory and absorptive functions of the GI epithelium, local blood flow and neuroimmune function (Kunze & Furness, 1999; Cooke, 2000). Both plexuses receive synaptic inputs from the sympathetic and parasympathetic divisions of the autonomic nervous system (Lundgren, 2000; Powley, 2000). Sensory neurons whose cell bodies are in spinal sensory and nodose ganglia can also release neurotransmitters, including ATP from their distal ends onto enteric neurons (Holzer, 2001; Kirkup *et al*., 2001). However, most synaptic input to enteric neurons comes from other enteric neurons (Kunze *et al*., 1993; Galligan *et al*., 2000).

There are two types of enteric neurons: 'S' neurons and 'AH' neurons (Kunze & Furness, 1999; Galligan *et al*., 2000). Electrical stimulation of interganglionic fiber tracts in the myenteric plexus elicits fast excitatory postsynaptic potentials (fEPSPs) in many S neurons, and the fEPSP is a prominent mechanism of synaptic excitation of S neurons (Nishi & North, 1973; Hirst *et al*., 1974). Acetylcholine and ATP are the principal fast excitatory synaptic transmitters in the ENS (Galligan *et al*., 2000). Data from electrophysiological, anatomical and functional studies indicate that S neurons are interneurons and motor neurons (Brookes, 2001). Some AH neurons also receive fast excitatory synaptic input (Grafe *et al*., 1979; Tamura & Wood, 1989; Liu *et al*., 1997; Cornelissen *et al*., 2001; Linden *et al*., 2003), but most of these neurons only receive slow excitatory synaptic input (Kunze *et al*., 1993; Furness *et al*., 1998).

The action potential in AH neurons has a prominent shoulder that is due to a calcium current. The action potential is followed by a slowly developing but long-lasting (1–20 s) afterhyperpolarization (Nishi & North, 1973; Hirst *et al*., 1974). The slow afterhyperpolarization is due to activation of a calcium-dependent potassium channel that is activated by

calcium entering the neuron during an action potential (Morita *et al*., 1982; Hirst *et al*., 1985). Recent work has shown that an intermediate-conductance (I_K) calcium-dependent potassium channel mediates the action potential slow afterhyperpolarization (Vogalis *et al*., 2002). Under resting conditions, AH neurons fire one or two action potentials as the afterhyperpolarization limits the firing rate. The function of AH neurons in the ENS is controversial, but these neurons may function as primary afferent neurons intrinsic to the gut wall (Kirchgessner & Gershon, 1988; Furness *et al*., 1998). The issues surrounding classification of AH neurons as intrinsic primary afferent neurons have been reviewed recently (Furness *et al*., 2004).

P2X receptors in the ENS

Overview of P2X receptors

There have been several recent reviews of the pharmacology and physiology of P2X receptors expressed in heterologous systems or in native tissues (Norenberg & Illes, 2000; North & Surprenant, 2000; Robertson *et al*., 2001; North, 2002). The reader is referred to these previously published papers for detailed information about the structure, function and pharmacology of P2X receptors. A brief overview of P2X receptor structure and function is provided here.

P2X receptors are ATP-gated cation channels. There are seven P2X receptor subunits ($P2X_{1-7}$) and each subunit has two membrane-spanning domains and the subunits may assemble as trimers to form functional P2X receptors (Jiang *et al*., 2003). Functional P2X receptors can be homomeric or heteromeric, and several subunit combinations form functional P2X receptors in heterologous expression systems. P2X receptors composed of different subunits or subunit combinations have unique pharmacological and functional properties (North & Surprenant, 2000), and these properties defined in heterologous systems can be used to help identify the subunit combinations composing P2X receptors in native cells. While a number of different subunit combinations can form functional P2X receptors in heterologous systems (Torres *et al*., 1999), the subunit combinations that are likely to co-assemble to form functional P2X receptors in native cells are more limited (North, 2002). For example, there is good evidence to indicate that $P2X_2$ and $P2X_3$ subunits co-assemble to form functional $P2X_{2/3}$ heteromeric receptors in a subset of dorsal root ganglion neurons (Lewis *et al*., 1995; North, 2002; Kennedy *et al*., 2003).

Pharmacology of enteric P2X receptors

The pharmacological properties of enteric P2X receptors have been characterized using enteric neurons maintained in primary culture (Barajas-Lopez *et al*., 1996; Zhou & Galligan, 1996). Pyridoxal phosphate 6-azophenyl-2′,4′-disulfonic acid (PPADS) is an antagonist that blocks some P2X receptors (North & Surprenant, 2000). In most neurons, ATP causes an inward current that desensitizes by more than 80% in 7 s. ATP-induced inward currents are blocked by PPADS but are not mimicked by α,β-methylene ATP (Zhou & Galligan, 1996). α,β-Methylene ATP is an agonist at P2X receptors containing $P2X_1$ and $P2X_3$ subunits but it does not activate $P2X_2$ homomeric receptors (North & Surprenant, 2000); so, P2X receptors on myenteric neurons may be $P2X_2$ homomeric receptors. The slow desensitization of the ATP response in myenteric neurons also suggests that it is mediated by P2X receptors that contain $P2X_2$ subunits (North, 2002). It is not known, yet, if these receptors in the guinea pig small intestine are $P2X_2$ homomers or are heteromers containing other P2X receptor subunits. This issue has been addressed, in part, in studies of P2X receptors in the small intestine of wild-type mice and in $P2X_2$ subunit gene knockout mice (Ren *et al*., 2003). In these studies, it was found that ATP but not α,β-methylene ATP depolarized S neurons in tissues from wild-type mice. ATP or α,β-methylene ATP did not depolarize S neurons from $P2X_2$ subunit knockout mice. These data indicate that in the mouse small intestine the P2X receptor expressed by S neurons is a $P2X_2$ homomeric receptor.

In approximately 10% of myenteric neurons maintained in primary culture, ATP elicited a rapidly developing inward current (10–90% rise time <50 ms) that desensitized in <200 ms. The rapidly desensitizing ATP response was mimicked by α,β-methylene ATP (Zhou & Galligan, 1996). These properties are similar to those of P2X receptors containing $P2X_3$ subunits (Lewis *et al*., 1995; North, 2002), but the subset of myenteric neurons expressing this receptor was not identified. Studies in small intestinal tissues from wild-type mice have shown that AH neurons are depolarized by ATP and α,β-methylene ATP (Bian *et al*., 2003). However, AH neurons in the small intestine from $P2X_3$ subunit knockout mice were depolarized by ATP but not by α,β-methylene ATP. These data indicate that P2X receptors in AH neurons are heteromeric receptors composed of $P2X_3$ subunits and an unidentified subunit.

$P2X_7$ receptor subunits have been localized to nerve terminals and nerve cell bodies in the ENS of guinea pig ileum (Hu *et al*., 2001) Homomeric $P2X_7$ receptors are relatively insensitive to activation by ATP ($EC_{50} = 100\,\mu$M), but they are activated by low concentrations ($EC_{50} = 3\,\mu$M) of the ATP analog 2-,3′-*O*-(4-benzoylbenzoyl) ATP (BzATP) (North, 2002). BzATP causes inward currents in myenteric neurons maintained in primary culture but the EC_{50} value is 250 μM (Hu *et al*., 2001) and ATP produces larger currents than BzATP at equimolar concentrations. KN-62 blocks $P2X_7$ receptors with an IC_{50} value of 50 nM (North & Surprenant, 2000); however, in myenteric neurons, KN-62 inhibits ATP-induced currents by 25% at 3 μM. $P2X_7$ homomeric receptors are pore-forming receptors that become permeable to large cations during prolonged activation (North, 2002). It is not known if enteric neuronal $P2X_7$ receptors expressed have this pore-forming capacity. $P2X_7$ receptors in the ENS have unique pharmacological properties that discriminate them from heterologously expressed receptors. Therefore, the $P2X_7$ subunit-containing receptors are probably not $P2X_7$ homomeric receptors. $P2X_7$ receptor subunits may combine with other P2X subunits in enteric neurons to form heteromeric receptors with unique pharmacological and functional properties.

P2X receptors mediate fast synaptic transmission

ATP acting on P2X receptors contributes to electrically evoked fEPSPs recorded from many myenteric S neurons in the guinea pig GI tract (LePard *et al*., 1997; Johnson *et al*., 1999; Monro *et al*., 2004; Nurgali *et al*., 2003). Therefore, drugs acting at P2X receptors on S neurons would modify synaptic excitation

of interneurons conveying synaptic information up and down the length of the gut and of motor neurons supplying the muscle layers. Modification of synaptic input to interneurons and motor neurons would be a useful mechanism of action for drug treatment of IBS. Identification of the specific subunit composition of P2X receptors mediating synaptic excitation of interneurons and motor neurons is needed prior to the development of drugs that would target these neurons.

Immunohistochemical studies of the guinea pig myenteric plexus showed that $P2X_2$ receptor subunits are expressed by nitric oxide synthase (NOS)-containing inhibitory motor neurons, descending interneurons and calbindin-containing neurons (Castelucci *et al*., 2002). NOS-containing inhibitory motor neurons participate in descending motor reflexes. Expression of $P2X_2$ subunits by neurons with descending projections is consistent with previous functional studies in which it was shown that neurons receiving non-cholinergic fast synaptic inputs were in descending pathways (Lepard & Galligan, 1999) and that NOS-containing inhibitory motor neurons receive P2X receptor-mediated fast synaptic input (Johnson *et al*., 1999). These data are consistent with the hypothesis that ATP acting at $P2X_2$ subunit-containing receptors contributes to synaptic excitation in descending motor pathways in the guinea pig intestine (Figure 1). This conclusion is supported by data obtained in tissues from $P2X_2$ subunit knockout mice (Ren *et al*., 2003). Although the functional class of neurons was not identified, it was shown that all fEPSPs were blocked completely by a nicotinic cholinergic receptor antagonist in the small intestine of $P2X_2$ receptor knockout mice. Therefore, fEPSPs were mediated only by acetylcholine acting at nicotinic receptors. ATP did not depolarize the same S neurons, but these neurons did respond normally to nicotine. In tissues from $P2X_2$ wild-type mice, fEPSPs were blocked only by the combined application of a nicotinic receptor antagonist and PPADS (Ren *et al*., 2003). Therefore, the P2X receptor expressed by myenteric S neurons in the mouse intestine is a $P2X_2$ homomeric receptor. However, studies of the guinea pig myenteric plexus showed that immunoreactivity for the $P2X_3$ subunit is expressed by NOS-containing inhibitory motor neurons (Poole *et al*., 2002; VanNassauw *et al*., 2002), indicating that $P2X_3$ subunits could contribute to P2X receptors mediating fEPSPs in descending pathways in the guinea pig small intestine. As discussed above, the non-cholinergic component of fEPSPs is absent in all myenteric S neurons from $P2X_2$ knockout mice, suggesting that only $P2X_2$ subunits compose the P2X receptor expressed by mouse myenteric S neurons. There may be differences between guinea pig and mouse in the subunit composition of functional P2X receptors expressed by S neurons. Alternatively, $P2X_3$ subunits in guinea pig S neurons may not contribute to the P2X receptor mediating fEPSPs in guinea pig ileum myenteric neurons. For example, the $P2X_3$ subunits might not be assembled into functional P2X receptors or $P2X_3$ subunits might be localized to extrasynaptic sites that are not accessible to synaptically released ATP.

When discussing potential targets for drugs to be used in the treatment of IBS, receptors on colonic myenteric neurons are an important site of action for these proposed agents. Electrophysiological have shown that 20–50% of myenteric neurons in the guinea pig colon receive fast synaptic input that is mediated partly by ATP acting at P2X receptors (LePard *et al*., 1997; Nurgali *et al*., 2003). The combined electrophysiological and morphological approach used by Nurgali *et al*. (2003) showed that there was no specific association of P2X-mediated fEPSPs with a functional class of S neuron. It appears then that P2X-mediated fEPSPs contribute to synaptic transmission in ascending and descending pathways in the guinea pig colon (Nurgali *et al*., 2003). This differs from the selective expression of P2X-mediated fEPSPs in descending projections in the guinea pig small intestine (see above).

Functional studies of P2X receptors on AH neurons

Myenteric and submucosal AH neurons with Dogiel type II cell morphology have processes that terminate in the mucosa of the intestine (Kirchgessner & Gershon, 1988; Song *et al*., 1994; 1998; Hens *et al*., 2000). As AH neurons may function as intestinal primary afferent neurons (Furness *et al*., 1998; 2004), substances released from epithelial cells or from enterochromaffin (EC) cells could act at receptors expressed by the nerve terminals of AH neurons in the mucosal layer (Bertrand & Bornstein, 2002). AH neurons also have processes that ramify in enteric ganglia, and this provides the anatomical substrate for activation of motor reflexes in response to mucosal stimulation (Kirchgessner & Gershon, 1988; Kunze *et al*., 1993; Kunze & Furness, 1999; Furness *et al*., 2004).

Most AH neurons contain the calcium-binding protein calbindin D28K (Furness *et al*., 1998), and these cell also express immunoreactivity for $P2X_2$ receptor subunits (Castelucci *et al*., 2002; VanNassauw *et al*., 2002). $P2X_2$ subunits expressed by AH neurons might mediate excitatory responses caused by ATP released by mucosal epithelial cells. Studies carried out in preparations of the guinea pig small intestine with the mucosa intact showed that application of ATP to mucosal villi caused antidromic action potentials that could be recorded in the cell body of myenteric AH neurons (Bertrand & Bornstein, 2002). ATP-induced responses were inhibited by a high concentration (60 μM) of PPADS, but were not inhibited by suramin nor were they mimicked by α,β-methylene ATP. These data suggest that ATP released in the mucosa by mechanical or chemical stimuli can activate AH neurons *via* an action at an unidentified P2X on the nerve cell body or nerve terminals. $P2X_4$ homomeric receptors are not activated by α,β-methylene ATP and are relatively insensitive to PPADS or suramin antagonism (North, 2002). Therefore, the P2X receptor expressed by guinea pig myenteric AH neurons could be a $P2X_4$ subunit-containing receptor. Immunohistochemical studies have shown that guinea pig AH type neurons express immunoreactivity for $P2X_2$ subunits, so the P2X receptors in these cells could be a $P2X_{2/4}$ heteromeric receptor. However, immunoprecipitation studies of epitope-tagged P2X receptor subunits expressed in HEK293 cells revealed that $P2X_2$ and $P2X_4$ subunits do not co-assemble to form a heteromeric complex (Torres *et al*., 1999). The subunit composition of functional P2X receptors in guinea pig AH neurons remains to be established (Figure 2).

α,β-Methylene ATP and ATP depolarize myenteric AH neurons in the mouse intestine. However, ATP but not α,β-methylene ATP depolarizes AH neurons in preparations from $P2X_3$ subunit knockout mice (Bian *et al*., 2003). Therefore, $P2X_3$ subunits contribute to the P2X receptors in murine AH neurons. As AH neurons in the small intestine of $P2X_3$ subunit knockout mice retain their sensitivity to ATP, other subunits must contribute to the receptor expressed by AH neurons. This

is a property that AH neurons share with some primary afferent neurons that express $P2X_{2/3}$ heteromeric receptors in dorsal root ganglia (Lewis *et al*., 1995; Kennedy *et al*., 2003). However, it is important to note that immunohistochemical studies in the guinea pig myenteric plexus have shown that $P2X_3$ subunits are not found in calbindin-containing AH-type neurons. There may be species differences in the expression of P2X receptor subunits by functional subsets of enteric neurons.

P2X receptors on submucosal plexus neurons

Neurons in the submucosal plexus control water and electrolyte secretion by GI epithelial cells (Cooke, 2000), and the volume of water and electrolytes absorbed from or secreted into the small intestinal or colonic lumen will impact on the volume of fecal content and defecation. Therefore, drugs that target receptors on submucosal neurons would have potential use in the treatment of IBS.

Immunoreactivity for $P2X_2$, $P2X_3$ and $P2X_7$ subunits has been localized to subsets of submucosal neurons in the guinea pig small intestine and colon (Hu *et al*., 2001; Castelucci *et al*., 2002; Poole *et al*., 2002). Exogenously applied ATP causes a rapidly developing depolarization or inward current in many submucosal neurons and these responses were blocked by suramin and PPADS (Barajas-Lopez *et al*., 1998; 2002). As in the myenteric plexus, ATP and acetylcholine are fast excitatory synaptic transmitters. However, in the guinea pig submucosal plexus, only about 20% of these neurons exhibit a component of the fEPSP mediated at P2X receptors (Monro *et al*., 2004) (Figure 1).

Contribution of P2X to intestinal reflexes

Studies of distention-evoked reflexes in an isolated segment of guinea pig intestine support a role for P2X receptors in mediating synaptic transmission in descending inhibitory motor pathways (Johnson *et al*., 1999; Bian *et al*., 2000). In these experiments, divided organ baths were used that isolated sites of drug application to either the site of distention, to a site that would block transmission between descending interneurons or to a site that would block synaptic input to the final inhibitory motor neurons. The nicotinic cholinergic receptor antagonist, hexamethonium, and the P2 receptor antagonists suramin and PPADS did not block transmission between interneurons in descending inhibitory reflex pathways (Johnson *et al*., 1999; Bian *et al*., 2000). However, PPADS blocked reflex-evoked inhibitory responses when applied to the site where inhibitory responses (relaxation or an inhibitory junction potential) were recorded. These data indicated that P2X-mediated synaptic excitation in descending inhibitory pathways occurs directly at the synapse between a descending interneuron and the inhibitory motor neurons (Johnson *et al*., 1999; Bian *et al*., 2000). P2X receptors also contribute to interneuronal transmission in descending excitatory pathways in the guinea pig small intestine (Spencer *et al*., 2000; Monro *et al*., 2002). Descending excitation is mediated by reflex activation of cholinergic motor neurons supplying the muscle layers. Descending excitatory neural inputs to the longitudinal and circular muscle caused by either wall distention or mucosal distortion are resistant to nicotinic cholinergic receptor antagonists. However, PPADS blocks reflex activation of both circular and longitudinal muscle layers, indicating that ATP acting on P2X receptors is the principal excitatory neurotransmitter in descending excitatory pathways in the guinea pig intestine (Spencer *et al*., 2000; Monro *et al*., 2002).

P2X on sensory nerves supplying the GI tract

Abdominal pain is a prominent symptom in IBS patients (Drossman *et al*., 2002). Therefore, one target for drug treatment of IBS would be sensory nerves conveying nociceptive information from the GI tract to the central nervous system. Nociceptive information from the GI tract is carried principally by spinal afferent neurons whose cell bodies are in dorsal root ganglia (Holzer, 2001; Blackshaw & Gebhart, 2002). Furthermore, dorsal root ganglion neurons express $P2X_2$ and $P2X_3$ receptor subunits (Lewis *et al*., 1995; Kennedy *et al*., 2003; North, 2003). The endings of spinal afferent nerve fibers supplying the small intestine also express functional P2X receptors, as local application of ATP or α,β-methylene ATP increases action potential firing rate of these nerve fibers (Kirkup *et al*., 1999). ATP and α,β-methylene ATP also sensitize mechanosensitive nerve endings in vagal afferent nerve fibers supplying the esophagus (Page *et al*., 2000). These data provide an anatomical and functional substrate for P2X receptors as targets for drugs that could modify pain signals arising in the gut wall (Figure 2). ATP released by epithelial cells in response to physiological stimuli (distention for example) or under pathophysiological conditions (noxious distention, inflammation) would activate P2X receptors on spinal afferents that would then convey feelings of distention, under physiological conditions, or pain in pathophysiological states (Holzer, 2001; Kirkup *et al*., 2001). Indeed, recent studies have shown that spinal afferent nerve fibers supplying the colorectum in the rat express $P2X_3$ receptor subunits. Balloon distention of the rectum causes local release of ATP, presumably from the mucosal layer, and locally released ATP activates afferent nerve fibers projecting from the colorectum to the lumbar–sacral portion of the spinal cord. (Wynn *et al*., 2003). These responses were prominent in high-threshold afferent fibers indicating that P2X receptors may participate in transmission of pain signals to the central nervous system from the colorectum in the rat (Wynn *et al*., 2003).

Future directions

The information derived from animal studies and summarized in this review indicates that P2X receptors are rational targets for drugs that could be used to treat IBS. $P2X_2$ receptors are expressed by non-enteric autonomic neurons and by neurons in the CNS. Currently, the best evidence for a functional role of $P2X_2$ receptors in synaptic transmission in an identified neural pathway is in the myenteric plexus of the guinea pig intestine (Bian *et al*., 2000; Khakh, 2001). Therefore, drugs acting at $P2X_2$ receptors might alter GI motility without disrupting synaptic mechanisms in other parts of the nervous system. This suggestion is supported by studies carried out in the small intestine of $P2X_2$ subunit knockout mice. These animals exhibited no behavioral or developmental abnormalities but peristalsis was impaired in the small intestine of these

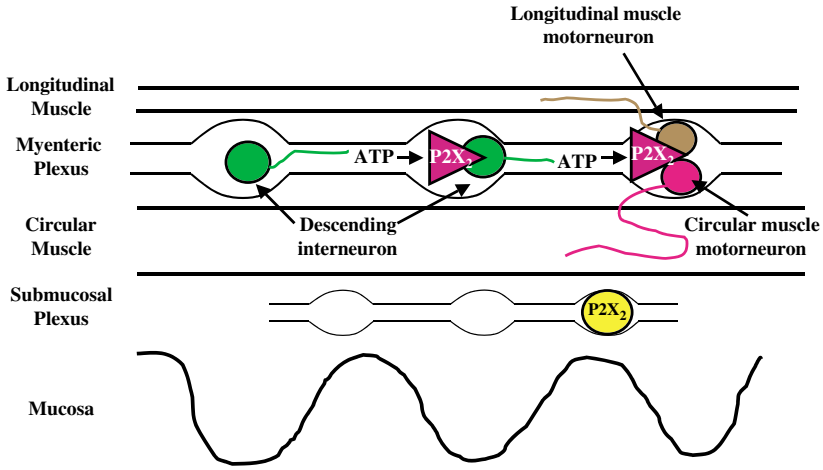

Figure 1 P2X receptors to descending interneurons and inhibitory motor neurons in the guinea pig small intestine. Analysis of the projections of neurons providing P2X-mediated synaptic input in the myenteric plexus has revealed that these projections are in descending pathways in the intestine. P2X receptors are localized to interneurons and motor neurons in these descending pathways. $P2X_2$ subunits contribute to the P2X receptors expressed by these neurons. P2X receptors are also found in submucosal neurons. These are also likely to contain of $P2X_2$ subunits.

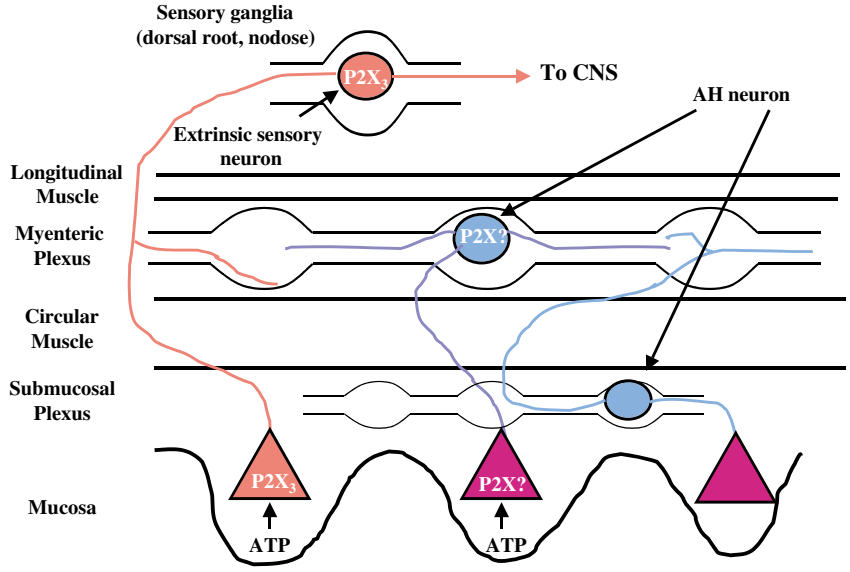

Figure 2 Localization of P2X receptors to primary afferent neurons intrinsic supplying the GI tract. The nerve cell body and nerve terminals of myenteric plexus AH-type neurons express P2X receptors, but the subunit composition of these receptors is unclear. It is not known if P2X receptors are found on the nerve terminals of submucosal plexus AH neurons. AH neurons can function as sensory neurons intrinsic to the wall of the intestine. $P2X_3$ receptors are localized to extrinsic primary afferents conveying nociceptive signals to the CNS.

mice (Ren *et al*., 2003). Based on these data, $P2X_2$ receptors appear to be promising target drugs that could selectively alter GI motility without disrupting synaptic transmission in other parts of the nervous system. So, antagonists acting at $P2X_2$ receptors would be expected to delay intestinal propulsion and these drugs would be useful in treating diarrhea-predominant IBS. Alternatively, drugs that facilitate $P2X_2$-mediated synaptic transmission might enhance intestinal propulsion and, therefore, they would be useful in treating constipation-predominant IBS. Future work in this area would be facilitated by the development of potent and selective ligands for $P2X_2$ homomeric receptors. To be useful, the $P2X_2$ selective ligands would need to retain their potency and selectivity *in vivo*.

P2X receptors are localized to intrinsic and extrinsic primary afferent neurons supplying the GI tract. Activation of P2X receptors on intrinsic and extrinsic primary afferent nerves would initiate propulsive motor reflexes in the GI tract. Activation of P2X receptors on extrinsic primary afferent nerve terminals might also contribute to transmission of nociceptive information to the CNS. Antagonists acting at primary afferent nerve terminal P2X receptors would be predicted to delay intestinal propulsion and to block ATP-mediated activation of extrinsic sensory nerves. Therefore, these drugs might be useful for the treatment of diarrhea-predominant IBS and associated visceral hypersensitivity and pain. P2X receptor antagonists have anti-nociceptive effects in human and animal models of acute and chronic pain (Bleehen

& Keele, 1977; Chizh & Illes, 2001; Kennedy *et al*., 2003; North, 2003). However, it has not yet been established if the same drugs are effective against GI pain or visceral hypersensitivity. Data indicating a role for P2X receptors in animal models of visceral hypersensitivity or GI pain are needed to provide further support for the development of P2X receptor-selective drugs for the treatment of visceral hypersensitivity in IBS. In addition, a careful characterization of the distribution of P2X receptor subtypes in human tissues, particularly the GI tract, is needed. Immunoreactivity for $P2X_3$ subunits have been localized to enteric neurons and also in the nerve terminals of extrinsic primary afferent neurons in samples of human colon (Facer *et al*., 2001; Yiangou *et al*., 2001). However, identification of the functional classes of human enteric neurons expressing $P2X_3$ and other P2X subunits is needed in order to understand the potential consequences of receptor activation or antagonism on human GI function. More extensive information about P2X subunit distribution in non-GI tissues in the human would also provide insights into potential adverse effects of P2X receptor ligands.

Immunohistochemical studies have localized P2X receptors to nerve fibers throughout the GI tract. The function of enteric neuronal terminal P2X receptors has not been studied systematically. In sympathetic neurons, activation of nerve terminal P2X receptors causes norepinephrine and ATP release (Sperlagh & Vizi, 1991; Boehm, 1999). Studies in synaptosomes prepared from the guinea pig myenteric plexus have shown that ATP can increase the release of acetylcholine from these isolated nerve terminals (Reese & Coupar, 1982). Further study of P2X receptors on enteric nerve endings would reveal if these receptors are potential targets for drug treatment of IBS.

The potential link between acute episodes of gastroenteritis and subsequent development of IBS in some patients has heightened interest in interactions between the immune system and the nerve supply of the GI tract (Sharkey & Kroese, 2001; Sharkey & Mawe, 2002; Spiller, 2003). P2X receptors, particularly $P2X_7$ receptors, are expressed by macrophages, lymphocytes and also by mast cells (reviewed by DiVirgilio *et al*., 2001; North, 2002). There have been no studies of the expression of $P2X_7$ receptors by immune cells or mast cells in the GI tract. It would be useful to characterize the distribution and function of $P2X_7$ receptors in immune cells in the gut. This information might provide the basis for a drug treatment strategy that would inhibit immune system-induced alterations in the function of enteric and afferent nerves supplying the GI tract.

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