Chapter 10
Purinergic Signalling in the Gut

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Introduction

Parasympathetic nerve stimulation that produced atropine-resistant responses of gastrointestinal smooth muscle was recognised early (Langley 1898; McSwiney and Robson 1929; Paton and Vane 1963). However, it was not until the early 1960s that gastrointestinal neuromuscular transmission other than that mediated by the classical transmitters acetylcholine (ACh) and noradrenaline (NA) was recognised (Burnstock et al. 1964; see Burnstock 1969, 2008a). The identification of adenosine 5′-triphosphate (ATP) as the non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmitter in the gut was proposed in 1970 (Burnstock et al. 1970) and the purinergic signalling hypothesis was launched in a Pharmacological Review (Burnstock 1972). This hypothesis was rejected by many people over the next 20 years and it was often ridiculed at international meetings (see Burnstock et al. 2010; Burnstock 2012a). Resistance to the concept was perhaps understandable, because ATP was established as an intracellular energy source involved in the Krebs cycle and it seemed unlikely that such a ubiquitous molecule would also act as an extracellular signaller. It is now clear that ATP, an ancient biological molecule, evolved both as an intracellular energy source and an extracellular signalling molecule. Later, after the cotransmitter hypothesis was published (see Burnstock 1976), it was recognised that nitric oxide (NO) and in some regions vasoactive intestinal polypeptide (VIP) were cotransmitters with ATP in NANC gastrointestinal inhibitory nerves.

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Strong evidence is now available in support of the purinergic hypothesis (see Olsson and Pearson 1990; Hoyle 1992; Dubyak and El Moatassim 1993; Zimmermann 1994; North 2002; Burnstock 2007a, 2012a; Burnstock et al. 2010; Burnstock and Verkhratsky 2012).

Intestinal motility, secretion and absorption can be influenced by ATP released from intrinsic enteric neurons, sympathetic nerves or sensory-motor nerves during axon reflexes, acting directly on purinoceptors on smooth muscle mediating relaxation or contraction or on epithelial cell secretion. Also, ATP released from mucosal epithelial cells can activate sensory enteric neurons involved in reflex activities. In addition, purine nucleotides and nucleosides can act on blood vessels, glia and interstitial cells of Cajal (ICCs) thereby indirectly modulating motility patterns. After breakdown to adenosine, ATP acts on prejunctional nerve terminals to modify transmitter release from motor and inhibitory neural pathways.

In the late 1980s and 1990s electrophysiological studies established that synaptic purinergic transmission was present between neurons in both myenteric and submucosal enteric plexuses (see LePard et al. 1997; Burnstock 2001a, 2007a; Galligan 2002; Bornstein 2008; Christofi 2008). The turning point for acceptance of purinergic signalling was when receptors for nucleotides and nucleosides were cloned and characterised in the early 1990s. Four subtypes of P1 (adenosine) receptors, 7 subtypes of P2X ion channel nucleotide receptors and 8 subtypes of G protein-coupled receptors were identified (see Ralevic and Burnstock 1998; Burnstock 2007b). RT-PCR and immunohistochemical studies were carried out to show the distribution of purinoceptor subtype mRNA and protein in different neurons and non-neuronal cells in different regions of the gastrointestinal tract of different species, including man (see Burnstock and Knight 2004; Burnstock 2007b, 2008a)

An exciting new field emerged when purinergic mechanosensory transduction in visceral organs was discovered (see Burnstock 1999, 2009). ATP released from mucosal epithelial cells during distension of the gut activates P2X3 receptors on submucosal sensory nerve endings (Wynn et al. 2003). Low threshold intrinsic sensory nerves mediate enteric reflex activity, while high threshold fibres mediate the initiation of pain via extrinsic sensory nerves.

There is increasing interest in the pathophysiology of purinergic signalling in the gastrointestinal tract (see Burnstock 2008b, 2014) and in this article its involvement in inflammatory bowel disease (IBD) (Yiangou et al. 2001; Wynn et al. 2004) will be considered.

The Early Discovery of Purinergic Neuromuscular Transmission

Correlated electrical and mechanical activity was recorded in the guinea pig taenia coli using the sucrose-gap technique (Burnstock and Straub 1958). After stimulation of the intramural nerves in the presence of adrenergic and cholinergic blocking

agents, hyperpolarisations and relaxations were reported (Burnstock et al. 1963, 1964; see Burnstock 2004). Tetrodotoxin, a neurotoxin that prevents the action potential in nerves without affecting the excitability of smooth muscle cells blocked the hyperpolarisations (Bülbring and Tomita 1967). This established them as inhibitory junction potentials (IJPs) in response to stimulation of NANC inhibitory nerves. Later NANC neurotransmission was shown to be mediated by intrinsic enteric neurons controlled by vagal and sacral parasympathetic nerves (Burnstock et al. 1966). NANC relaxations were identified at about the same time in the stomach upon stimulation of the vagus nerve (Martinson and Muren 1963; Martinson 1965).

The next step was to try to identify the transmitter released during NANC inhibitory transmission in the gut. Several criteria were postulated by Eccles (1964) and also by Paton (1958) that needed to be satisfied to establish a neurotransmitter: synthesis and storage in nerve terminals; release by a Ca^{2+}-dependent mechanism; mimicry of the nerve-mediated responses by the exogenously applied transmitter; inactivation by ectoenzymes and/or neuronal uptake; and parallel block of responses to stimulation by nerves and exogenously applied transmitter. Different substances were examined in the late 1960s, including amino acids, monoamines and neuropeptides, but none satisfied the criteria. However, hints in a paper by Drury and Szent-Györgyi (1929) showing extracellular actions of purines on heart and blood vessels, a paper by Feldberg and Hebb (1948) showing extracellular actions of ATP on autonomic ganglia and a paper by Holton (1959) showing release of ATP during antidromic stimulation of sensory nerves supplying the rabbit ear artery, led Burnstock and his colleagues to consider ATP and this satisfied all the criteria required to establish it as a transmitter involved in NANC inhibitory neurotransmission (Burnstock et al. 1970). The purinergic neurotransmission hypothesis was proposed in a Pharmacological Review in 1972 (Burnstock 1972).

There was early evidence for ATP as a cotransmitter in sympathetic nerves supplying the guinea pig taenia coli (Su et al. 1971). Periarterial sympathetic nerve stimulation led to release of tritium from guinea pig taenia coli preincubated in [^{3}H] adenosine (which was taken up and converted largely to [^{3}H]ATP) and the release of both tritium and NA was blocked by guanethidine. The proportion of ATP and NA in sympathetic nerves varies in different regions of the gut, between species and during development and ageing. It has been reported that ATP is the sole transmitter in sympathetic nerves supplying arterioles in the submucosal plexus of the intestine, while NA released from these nerves acts as a prejunctional modulator of transmitter release (Evans and Surprenant 1992). 'Axon reflex' activity involving sensory-motor nerves is widespread in autonomic effector systems and forms an important physiological component of autonomic control of blood vessels and visceral organs, including the gut (Burnstock 1993; Holzer 2006). ATP and glutamate are cotransmitters in primary afferent sensory nerves. Cotransmission occurs in enteric neurons and the concept of 'chemical coding' was proposed as a consequence of the patterns of colocalisation of neuropeptides defining specific neuron types (Furness et al. 1989). It is now recognised that three major cotransmitters are released from NANC inhibitory enteric nerves: (1) ATP producing fast IJPs; (2) NO also eliciting IJPs, but with

a slower time course; and (3) VIP producing slow tonic relaxations (Burnstock 2001a). In some sphincters the NANC inhibitory nerves primarily utilise VIP, in others they utilise NO, and in non-sphincteric regions of the intestine, ATP is prominent. Detailed accounts of purinergic neuromuscular transmission in different regions of the gut are available (Hoyle and Burnstock 1989; Burnstock 2001a, 2014; Burnstock and Verkhratsky 2012).

NANC inhibitory purinergic transmission to intestinal smooth muscle of laboratory animals and humans is mediated by $P2Y_1$ receptors (Wang et al. 2007; Gallego et al. 2008, 2012). α,β-Methylene ATP (α,β-meATP) has a potent relaxant action in some preparations (Johnson and Hourani 1994; Johnson et al. 1996; Pacaud et al. 1996). However, it is likely that α,β-meATP is acting on P2X3 receptors on sensory nerves (Storr et al. 2000; De Man et al. 2003) leading to reflex activation of NANC inhibitory nerves and to $P2Y_1$ receptor-mediated relaxation of smooth muscle (see King and Townsend-Nicholson 2008). Evidence was presented that ATP mediates a non-cholinergic component of the excitatory junction potential and contraction of intestinal smooth muscle (Zagorodnyuk and Maggi 1998). ATP stimulated cholinergic interneurons in the myenteric plexus to cause a fast contraction of rat ileum (Sakai et al. 1979). $P2Y_2$ and/or $P2Y_4$ receptors were shown to mediate smooth muscle contractions in the small intestine of lower vertebrates (Burnstock 1969; Sneddon et al. 1973). Contraction of rat duodenal muscularis mucosae smooth muscle was mediated by P2X receptors (Johnson et al. 1996). P2X receptors mediated contraction of guinea pig ileum (Moody and Burnstock 1982; Ivancheva et al. 2001). Ileal contractions mediated by α,β-meATP were inhibited in P2X1 receptor knockout mice (Vial and Evans 2001). mRNAs for P2X2, P2X3 and P2X4 receptors were expressed by canine colon circular myocytes, while longitudinal myocytes expressed mRNAs for P2X3 and P2X5 receptors (Lee et al. 2005).

Synaptic Purinergic Transmission in the Enteric Plexuses

Elegant electrophysiological studies, carried out during the past 20 years have demonstrated synaptic purinergic transmission between enteric neurons in both myenteric and submucous plexuses in both in situ and tissue culture preparations (see Galligan et al. 2000; Galligan 2002; Hu et al. 2003; Ren et al. 2003; Galligan and North 2004; Ren and Galligan 2005; Burnstock 2007a, 2008a; Bornstein 2008; Ren and Bertrand 2008; Valdez-Morales et al. 2011). Also, extensive immunostaining of the localisation of both P2X and P2Y receptor subtypes in the gastrointestinal tract of guinea pigs, rats and mice was carried out (Gröschel-Stewart et al. 1999; Giaroni et al. 2002, 2006; Van Nassauw et al. 2002, 2006; Xiang and Burnstock 2004a, b, 2005, 2006; Ruan and Burnstock 2005; Yu et al. 2010).

Myenteric Ganglia

The effects of ATP in single myenteric neurons from guinea pig small intestine using intracellular electrodes were first shown by Katayama and Morita (1989). They showed that ATP produced hyperpolarisation in 80 % of AH neurons and depolarisation in 90 % of S neurons. The studies of purinergic signalling in guinea pig myenteric neurons were extended by several groups. Whole-cell and outside-out patch clamp recordings were used to characterise the physiological and pharmacological features of P2X receptors on myenteric neurons of the guinea pig ileum (Barajas-López et al. 1996). Fast excitatory postsynaptic currents (fEPSCs) were recorded in primary cultures of myenteric neurons from guinea pig intestine and hexamethonium-resistant fEPSCs were abolished by the P2 receptor antagonist pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) (Zhou and Galligan 1996; LePard et al. 1997). Fast excitatory postsynaptic potentials (EPSPs) were mediated in part by P2X receptors in myenteric neurons in both the small and large intestine, but were rare in the gastric corpus (LePard et al. 1997). P2X2 receptors were the dominant subtype shown to be expressed by subpopulations of guinea pig enteric neurons, namely inhibitory motor neurons, vasomotor neurons, cholinergic secretomotor neurons, intrinsic sensory neurons and the endings of vagal afferent fibres in the stomach (Castelucci et al. 2002; Misawa et al. 2010). Using P2X2 receptor knockout mice it was shown that P2X2 receptors contributed to fast synaptic excitation of myenteric neurons in small intestine (Ren et al. 2003). The predominant receptors mediating fast synaptic excitation in the gut appear to be P2X2 homomeric receptors (Galligan 2002; Galligan and North 2004; Ohta et al. 2005), including intrinsic sensory neurons in the gut (Furness et al. 2004b).

P2X3 receptors were expressed by both excitatory and inhibitory motor neurons, ascending interneurons and cholinergic secretomotor neurons (Poole et al. 2002). However, it was claimed that they were not expressed by intrinsic sensory neurons in guinea pig ileum (Van Nassauw et al. 2002). In the small intestine of mice lacking P2X3 receptors peristalsis was impaired (Bian et al. 2003). The distribution of the mRNA and protein of P2X2 and P2X3 receptors were described in the enteric nervous system of the rat (Xiang and Burnstock 2004b). Most myenteric S neurons in guinea pig small intestine expressed P2X3 receptors, about half of which were inhibitory motoneurons (Ren and Galligan 2007). Nerve fibres that enveloped ganglion cell bodies in the myenteric and submucous plexuses in mouse intestine expressed P2X5 receptors, probably as heteromultimers with P2X2 receptors on enteric sensory neurons (Ruan and Burnstock 2005).

Purinergic signalling in dispersed primary cultures of guinea pig myenteric plexus was studied by the group of Mulholland. Different populations of enteric neurons responded to combinations of ATP with ACh, ATP with substance P (SP), ATP with ACh, ATP with ACh and SP, ATP with bombesin or ATP with ACh and bombesin (Kimball and Mulholland 1995). When ACh and ATP acted as cotransmitters, there was an interaction between nicotinic and P2X receptors with

cross-inhibition between α3β4 nicotinic receptors and the C-terminal tail of P2X2 receptors (Decker and Galligan 2010). Inhibitory interactions have also been shown to take place between P2X and γ-aminobutyric acid-A receptors on myenteric neurons from the guinea pig small intestine (Karanjia et al. 2006). In excitatory neuro-neuronal transmission in both ascending and descending reflex pathways to the longitudinal and circular muscles of the guinea pig ileum triggered by mucosal stimulation, a major role was played by ATP (Clark et al. 1996; Spencer et al. 2000). P2X receptor-mediated transmission from interneurons to motor neurons in guinea pig ileum underlies descending inhibitory reflexes (Bian et al. 2000; Bornstein et al. 2004).

There is expression of P2Y receptors on enteric neurons in addition to P2X receptors (Xiang and Burnstock 2005, 2006; Van Nassauw et al. 2005; Gao et al. 2006; Wood 2006). In the mouse gastrointestinal tract relaxation is mediated by $P2Y_1$ receptors on NANC myenteric neurons (Giaroni et al. 2002). Slow excitatory synaptic transmission on S-type neurons in the guinea pig enteric nervous system was mediated by $P2Y_1$ receptors (Hu et al. 2003). They also mediated slow excitatory synaptic potentials on interneurons during descending inhibition in guinea pig ileum (Thornton et al. 2013). $P2Y_2$ receptors were expressed by S-type neurons in both myenteric and submucosal plexuses of the guinea pig gut. 40–60 % of P2X3 receptor-immunoreactive neurons were immunoreactive for $P2Y_2$ receptors in the myenteric plexus and all P2X3 receptor-immunoreactive neurons expressed $P2Y_2$ receptors in the submucosal plexus (Xiang and Burnstock 2005). 30–36 % of neurons in ganglia in the myenteric, but not submucosal plexus of the guinea pig gut expressed $P2Y_6$ receptors, while 42–46 % of the neurons in both myenteric and submucosal plexuses were immunoreactive for $P2Y_{12}$ receptors (Xiang and Burnstock 2006). 28–35 % of $P2Y_6$ receptor-immunoreactive neurons coexisted with NO synthase, while all $P2Y_{12}$ receptor-immunoreactive neurons were immunopositive for calbindin, on AH intrinsic sensory neurons. $P2Y_2$ and $P2Y_{12}$ receptors were identified on enteric neurons in the rat distal colon (Van Nassauw et al. 2005). Presynaptic A_1 receptors mediated suppression of slow EPSPs and amplified slow inhibitory postsynaptic transmission to myenteric neurons (Christofi and Wood 1993; Kamiji et al. 1994).

Submucosal Ganglia

The non-reversing type of slow excitatory postsynaptic potential recorded in S neurons of the submucous plexus of the guinea pig caecum was mimicked by ATP (Mihara et al. 1985). ATP produced fast transient depolarisation of AH-type neurons (Barajas-López et al. 1994), mediated by P2X receptors (Barajas-López et al. 2000). Neurons in the submucous plexus were immunopositive for P2X3 receptors and were colocalised with calretinin, suggesting labelling of intrinsic sensory neurons (Xiang and Burnstock 2004b). Functional interactions between nicotinic and

P2X receptors were demonstrated in dissociated guinea pig submucosal neurons in primary culture (Glushakov et al. 1996; Barajas-López et al. 1998; Zhou and Galligan 1998). Inhibitory interactions between P2X and 5-HT_3 receptors in guinea pig submucosal neurons were reported (Barajas-López et al. 2002). Slow, fast and intermediate EPSPs were recorded in neurons of the submucous plexus of the guinea pig ileum (Monro et al. 2004). The slow and intermediate EPSPs were blocked by the $P2Y_1$ receptor selective antagonist MRS2179. $P2Y_1$ receptor signalling involved in synaptic transmission in the human submucous nerve plexus was reported to be predominant (Wunderlich et al. 2008).

Intrinsic Sensory Neurons

Intrinsic sensory neurons are located in the submucosal and myenteric ganglia and their terminals are largely in a subepithelial plexus (Furness et al. 2004a). Intrinsic sensory neurons have been identified electrophysiologically as AH-type and morphologically as Dogiel type II cells. Most AH cells express calbindin and/or calretinin. Synaptic transmission to intrinsic sensory neurons is mediated by P2X receptors (Bertrand and Bornstein 2002), of the P2X2 receptor subtype in guinea pig intestine (Castelucci et al. 2002). Postsynaptic inhibition via P2Y receptors has also been identified on intrinsic sensory nerves (Bertrand 2003, 2004). P2X3 receptors were shown to be expressed by intrinsic sensory nerves in rat ileum and distal colon (Xiang and Burnstock 2004b). $P2Y_{12}$ receptors were expressed by sensory neurons in guinea pig myenteric plexus (Xiang and Burnstock 2006).

Enteric Glial Cells and Interstitial Cells of Cajal

Enteric glial cells respond to ATP and uridine 5′-triphosphate, increasing intracellular calcium via $P2Y_2$ and/or $P2Y_4$ receptors (Kimball and Mulholland 1996; Sarosi et al. 1998). Immunohistochemical studies also showed expression of P2X7 receptors on enteric glial cells (Vanderwinden et al. 2003) and $P2Y_4$ receptors (Van Nassauw et al. 2006). It was proposed that ATP released from sympathetic nerves activates enteric glia (Gulbransen et al. 2010). Purinergic neuron-glia interactions in the enteric nervous system have been reported, reflecting similar mechanisms in the CNS (Gulbransen and Sharkey 2009). From an electrophysiological study of a mouse enteric neuron-glial culture preparation, it was concluded that neuronal cells primarily express P2X receptors, while glial cells primarily express P2Y receptors (Gade and Akbarali 2013).

ICCs are a specialised cell type that act as pacemakers to regulate the activities of smooth muscle cells in the gut. P2X2 and P2X5 receptors were shown to be expressed on ICC's in guinea pig intestine (Burnstock and Lavin 2002). Later, $P2Y_4$ receptors were also identified on ICCs in guinea pig gastrointestinal tract

(Van Nassauw et al. 2006). It is likely that ATP is released as a cotransmitter from enteric nerves and glial cells to regulate the activities of ICCs (Burnstock and Lavin 2002). Modulation of pacemaker $[Ca^{2+}]_i$ activity in ICC's was mediated by P2X receptors (Furuzono et al. 2005). It was reported that ICCs in human and murine small intestine expressed $P2Y_1$ and $P2Y_4$ receptors (Chen et al. 2007). 'Fibroblast-like cells', that form a network of cells distinct from ICCs, located between intestinal circular and longitudinal smooth muscle near terminals of enteric motor neurons and with gap junction connectivity with muscle cells, express $P2Y_1$ receptors (Kurahashi et al. 2011). $P2Y_1$ receptor antagonists blocked the activation of currents and increase in $[Ca^{2+}]_i$ by adenosine 5′-diphosphate in these cells. The majority of subserosal ICCs or perhaps fibroblast-like cells in the guinea pig proximal colon responded to ATP via $P2Y_1$ receptors and it was suggested that this may contribute to smooth muscle relaxation (Tamada and Hashitani 2014).

Purinergic Mechanosensory Transduction: Enteric Reflexes and Pain

Both submucosal intrinsic sensory neurons and extrinsic sensory nerves show positive immunoreactivity for P2X3 receptors (Xiang and Burnstock 2004b). It has been proposed that during intestinal distension ATP is released from mucosal epithelial cells to activate P2X3 receptors on both low threshold enteric sensory nerve fibres to mediate enteric reflexes (including peristalsis) and high-threshold extrinsic enteric sensory fibres leading to initiation of nociceptive impulses that pass messages through sensory ganglia to pain centres in the CNS (Burnstock 2001b, 2009). This hypothesis has been supported by experiments on a rat pelvic sensory nerve-colorectal preparation (Wynn et al. 2003). Distension of the colorectum led to increase in release of ATP from mucosal epithelial cells and evoked pelvic sensory nerve excitation. This excitation was mimicked by application of ATP and was attenuated by the selective P2X3 and P2X2/3 antagonist, 2′(3′)-O-(2,4,6-trinitrophenyl) ATP, and by PPADS. The sensory activity in the nerves was potentiated by ARL-67156, an ATPase inhibitor. It has been claimed recently that subepithelial fibroblasts in rat ductal villi also release ATP by mechanical stimuli to activate P2X3 receptors on subepithelial sensory nerves (Furuya and Furuya 2013).

Purinergic Signalling in Inflammatory Gut Disorders

P2X3 receptors are upregulated on enteric sensory neurons in inflammation and hypersensitivity (Wynn et al. 2004). Intestinal inflammation also increased the expression of $P2Y_6$ receptors on epithelial cells and uridine diphosphate, a potent

$P2Y_6$ receptor agonist, released CXCL8, a chemokine known for chemoattraction to recruit neutrophils during the acute phase of colitis (Grbic et al. 2008, 2012). ATP may be beneficial in the treatment of intestinal disorders where intestinal permeability changes are involved (Bours et al. 2007). It was reported that $P2Y_2$ receptor expression was upregulated in intestinal epithelial cells by the transcription factor C/EBPβ during inflammation (Degagné et al. 2012).

Expression of P2X3 receptors was increased in enteric plexuses of human IBD, suggesting a role in dysmotility and pain (Yiangou et al. 2001) and the possibility that P2X receptor antagonists could be used for the treatment of irritable bowel syndrome (IBS) was raised (Galligan 2004). It was also suggested that P2X receptors on intrinsic enteric neurons may mediate enhanced gastrointestinal propulsion and secretion and might be used for treating constipation-predominant IBS, while P2X receptor antagonists might be useful for treating diarrhoea-predominant IBS. It has been suggested that sensitisation of P2X3 receptors on vagal and spinal afferents in the stomach may contribute to the development of visceral hyperalgesia (Dang et al. 2005). In inflamed gastrointestinal tract, glial cells proliferate and produce cytokines, indicating that P2X7 receptors may play a role in the response of enteric glia to inflammation (Vanderwinden et al. 2003).

ATP release and P2X3 and P2X2/3 receptor-mediated nociceptive sensory nerve responses were enhanced in the rat trinitrobenzene sulfonic acid (TNBS) model of colitis (Wynn et al. 2004). Different mechanosensory information from the colon to the spinal cord is mediated by lumbar splanchnic (LSN) and sacral pelvic (PN) nerves. It was shown that 40 % of LSN afferents responded to α,β-meATP compared to 7 % of PN afferents (Brierley et al. 2005). There is enhancement of P2X3 receptor-mediated signalling in the TNBS colitis model, which was due, at least in part, to the appearance of P2X3 receptor expression in a greater number of calcitonin gene-related peptide-labelled small nociceptive neurons in the dorsal root ganglia (DRG) (Wynn et al. 2004). There is also increased release of ATP from mucosal epithelial cells with distension in TNBS-treated rats. Purinergic mechanosensory transduction has been shown to contribute to post-infectious mechano-hypersensitivity (Rong et al. 2009). In TNBS-induced colitis in mice, P2X1 receptor expression on colonic submucosal arterioles was increased (Lomax et al. 2007). Propulsive motility was attenuated in the ulcerated region of the TNBS-inflamed colon and this was associated with a decrease in the purinergic component of the descending inhibitory limb of the peristaltic reflex circuit (Strong et al. 2010).

Substances are released from mucosal epithelial cells during distension that often act synergistically to cause sensitisation of afferent nerves to mechanical or chemical stimuli (Wynn and Burnstock 2006). Thus, receptors to a variety of substances including ATP are potential targets for drug treatment for inflamed bowel function and visceral pain (see Kirkup et al. 2001; Holzer 2004). The sensitising effects of P2X3 receptor agonists on mechanosensory function were also demonstrated in oesophagitis (Page et al. 2000). Visceral hyperalgesia was shown to be

associated with an increase in ATP activity and enhanced expression of P2X3 receptors in colonic sensory neurons (Xu et al. 2008). Selective P2X3 and P2X2/3 receptor antagonists that are orally bioavailable and do not degrade in vivo are in clinical trials for the treatment of visceral pain (see Gever et al. 2006; Donnelly-Roberts et al. 2008). P2X3 receptor mRNA expression in DRG was significantly decreased in an ovariectomized rat model of colitis, which was reversed by oestrogen (Fan et al. 2009). It was suggested that ATP is a critical autocrine regulator of mechanosensitive 5-hydroxytryptamine (5-HT) release, also involved in the pathogenesis of IBD and it was shown that P2X3 receptors on enterochromaffin cells were downregulated in ulcerative colitis (Liñán-Rico et al. 2013). CD39 (NPTDase 1) was upregulated in the submucosa during colitis, resulting in compromise of epithelial barrier function (Neshat et al. 2009). It was reported that dysregulation occurs in 59 % of purinoceptor genes in IBD, including $P2Y_6$, $P2Y_{13}$, $P2Y_{14}$, P2X5, A_{2A} and A_{2B} receptors (Rybaczyk et al. 2009).

P2X7 receptors play a pivotal role in intestinal inflammation and are involved in the development of visceral hypersensitivity (Keating et al. 2011). Epithelial and immune cells express P2X7 receptors, which are implicated in the pathogenesis of IBD based on the dysregulation of immune responses in (de Campos et al. 2012). Activation of neuronal P2X7 receptor/pannexin 1 mediates death of enteric neurons during colitis (Gulbransen et al. 2012). This supported an earlier study of TNBS-induced colitis, using high-density oligonucleotide microassay analysis and oral N^6-(3-iodobenzyl)-adenosine-5-N-methyluronamide, an A_3 receptor agonist, blocked the colitis-induced upregulation of P2X1, P2X4, P2X7, $P2Y_2$ and $P2Y_6$ receptors (Guzman et al. 2006). Extracellular ATP largely via P2X7 receptors evoked cell death in human intestinal epithelial cells and the implication of this in inflammatory conditions and immune responses was explored (Souza et al. 2012). It has also been shown that ATP mediated mast cell-dependent intestinal inflammation via P2X7 receptors (Kurashima et al. 2012).

An adenosine A_3 agonist has been recommended to be protective in two murine models of colitis (Mabley et al. 2003). A_{2B} receptor expression and signalling in intestinal epithelium in colitis was upregulated by tumour necrosis factor-α (Kolachala et al. 2005). A_{2B} receptor blockade ameliorated mouse colitis (Kolachala et al. 2008a) as did A_{2B} receptor gene deletion (Kolachala et al. 2008b). The inhibitory effects of adenosine on enteric neuromuscular activities were diminished in inflamed colon (Antonioli et al. 2005). Oxidative stress disrupted purinergic neuromuscular transmission in the inflamed colon (Roberts et al. 2013). A_{2B} receptors appear to play a role in the control of T cell-mediated colitis by suppressing the expression of pro-inflammatory cytokines, while sparing anti-inflammatory activity mediated by interleukin (IL)-10 and transforming growth factor-β (Naganuma et al. 2006). A_{2A} receptors were also reported to mediate the inhibitory effects of adenosine on colonic motility in the TNBS model of experimental colitis (Antonioli et al. 2006; Rahimian et al. 2010). Adenosine deaminase inhibition attenuates

inflammation in experimental colitis (Antonioli et al. 2007) through the recruitment of A_{2A} and A_3 receptors (Antonioli et al. 2010). Adenosine, acting via A_3 receptors, has been shown to be involved in intestinal anti-inflammation activities (Guzman et al. 2006; Gessi et al. 2008). A_{2A} receptors were also involved in the anti-inflammatory actions of adenosine (Odashima et al. 2005) and A_{2A} receptor agonists are being developed for the treatment of IBD (El-Tayeb et al. 2011). A_{2B} receptors mediate regulation of 5-HT synthesis and release from hypoxic enterochromaffin cells in IBD (Damen et al. 2013). A_{2B} receptor antagonists were reported to be effective against murine colitis (Kolachala et al. 2008c). The involvement of adenosine A_1 and A_{2A} receptors (Antonioli et al. 2011) and A_3 receptors (Ren et al. 2011) in colitis has been reported. Recent reviews of the roles of adenosine signalling in gastrointestinal inflammation are available (Estrela and Abraham 2011; Colgan et al. 2013). The involvement of adenosine deaminase in patients with Crohn's disease has been explored (Maor et al. 2011). Adenosine kinase inhibition by GP515 has also been investigated as a potential target for the treatment of colitis (Siegmund et al. 2001). It was concluded in a review about purinergic receptors in gastrointestinal inflammation (Kolachala et al. 2008a) that P1 (A_{2A} and A_{2B}) and P2Y receptor-based therapy is highly promising for treatment of inflammatory conditions of the gut (see Michael et al. 2010). Serum adenosine deaminase activity has been proposed as a predictor of disease severity in ulcerative colitis (Beyazit et al. 2012). Ecto-nucleoside triphosphate diphosphohydrolase 7 was preferentially expressed in epithelial cells of mouse small intestine (Kusu et al. 2013). ATP released from colonic mucosal epithelial cells of IBS patients excited via P2X receptors enteric cholinergic motor neurons (Balestra et al. 2012). The role of adenosine as an immune modulator of IBD has been considered (Ye and Rajendran 2009). Polymorphisms of CD39 have been linked to Crohn's disease (Künzli et al. 2011). Release of ATP by activated neutrophils and necrotic intestinal epithelial cells stimulates epithelial cell P2X7 receptors leading to activation of caspase 1 and secretion of IL-1β proinflammatory cytokine (Cesaro et al. 2010).

Concluding Comments

In this brief review, the focus has been on purinergic neuromuscular transmission, synaptic purinergic transmission in the enteric nerve plexuses, purinergic mechanosensory transduction in initiation of enteric reflexes and intestinal nociception and the involvement of purinergic signalling in inflammatory gut disorders. Figure 10.1 summarises the complex distribution of purinoceptor subtypes in the gut. For fuller coverage of the involvement of purinergic signalling in the physiology and pathophysiology of the gastrointestinal system, readers are recommended to refer to the following recent reviews (Burnstock 2008a, b, 2011, 2012b, 2014).

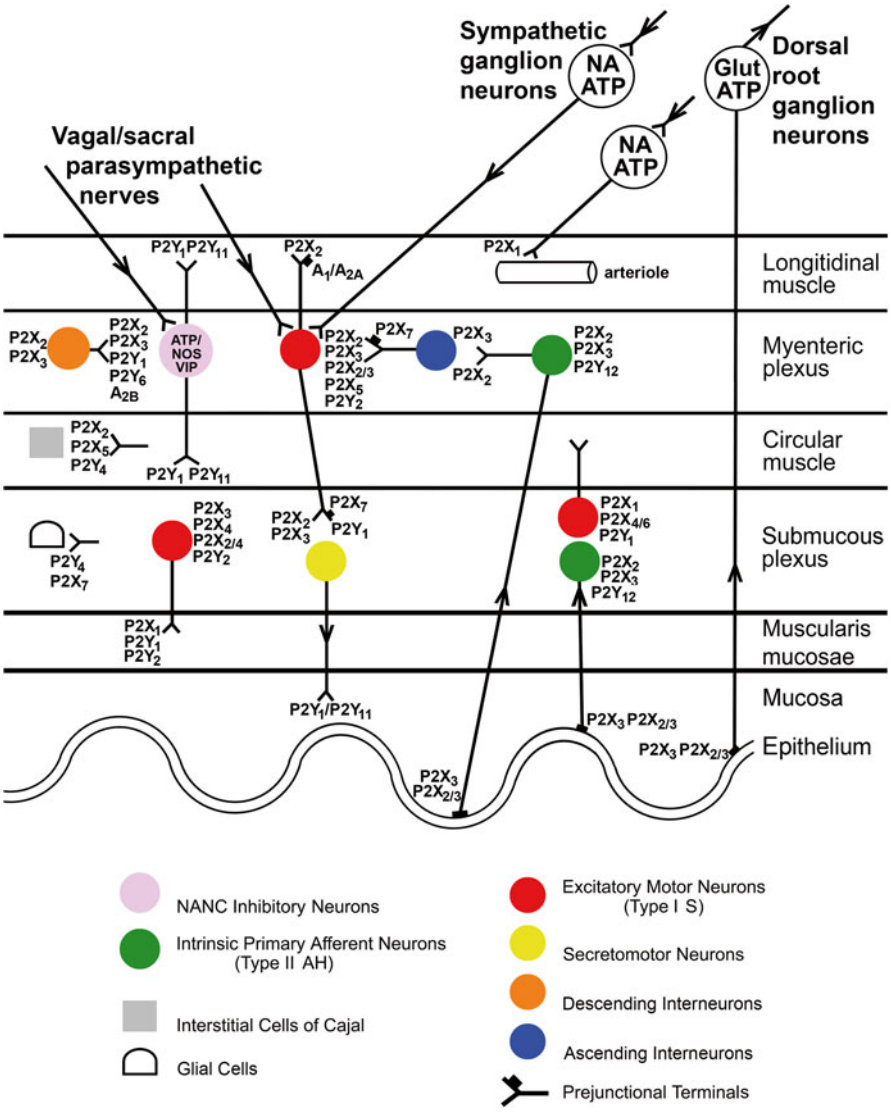

Fig. 10.1 Schematic showing the localisation of receptors to purines and pyrimidines on neurons and non-neuronal effector cells in the gut, although some of the interacting pathways are not yet known. Extrinsic vagal and sacral parasympathetic nerves connect with NANC inhibitory neurons in the myenteric plexus expressing P2X2, P2X3, $P2Y_1$, $P2Y_6$ and A_{2B} receptors, as well as with cholinergic motor neurons; these neurons are also activated by descending interneurons. Extrinsic sympathetic nerves modulate motility via excitatory motor neurons and constrict blood vessels in the gut via P2X1 receptors. Extrinsic sensory nerves arising from cell bodies in dorsal root ganglia and with subepithelial terminals mediate nociception. Intrinsic sensory neurons in both myenteric and submucosal plexuses express P2X2 and P2X3 receptors, while a subpopulation also express $P2Y_{12}$ receptors; they connect with motor pathways involved in peristalsis. Excitatory motor neurons express P2X2, P2X3, P2X2/3, P2X5 and $P2Y_2$ receptors and connect with both interneurons and secretomotor neurons. Interneurons express P2X2 and P2X3 receptors. Enteric glial cells express $P2Y_4$ and P2X7 receptors, while interstitial cells of Cajal express P2X2, P2X5 and $P2Y_4$ receptors. P2X7 and P1 receptors appear to act as prejunctional modulators of both motor and interneurons. (Reproduced from Burnstock 2008b, with permission.)

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