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REVIEW

Purinergic receptors and synaptic transmission in enteric neurons

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Abstract Purines such as ATP and adenosine participate in synaptic transmission in the enteric nervous system as neurotransmitters or neuromodulators. Purinergic receptors are localized on the cell bodies or nerve terminals of different functional classes of enteric neurons and, with other receptors, form unique receptor complements. Activation of purinergic receptors can regulate neuronal activity by depolarization, by regulating intracellular calcium, or by modulating second messenger pathways. Purinergic signaling between enteric neurons plays an important role in regulating specific enteric reflexes and overall gastrointestinal function. In the present article, we review evidence for purine receptors in the enteric nervous system, including P1 (adenosine) receptors and P2 (ATP) receptors. We will explore the role they play in mediating fast and slow synaptic transmission and in presynaptic inhibition of transmission. Finally, we will examine the molecular properties of the native receptors, their signaling mechanisms, and their role in gastrointestinal pathology.

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Abbreviations

2-Me-S-ATP	2-Methylthio-ATP
5-HT	5-Hydroxytryptamine or serotonin
α,β-m-ATP	α,β-Methylene ATP
ATP	Adenosine triphosphate
AH	Afterhyperpolarization
AHP	Afterhyperpolarizing potential
AH neuron	Type of neuron with a long-lasting AHP following the action potential
AP	Action potential
ChAT	Choline acetyl transferase
ENS	Enteric nervous system
EPAN	Extrinsic primary afferent (sensory) neuron—a vagal or spinal afferent innervating the GI tract with a cell body in the nodose or dorsal root ganglia
EPSP	Excitatory post-synaptic potential
GI tract	Gastrointestinal tract
IPAN	Intrinsic primary afferent neuron with a cell body in the wall of the gut, also called an intrinsic sensory neuron
NPY	Neuropeptide Y
NOS	Nitric oxide synthase
RMP	Resting membrane potential
PPADS	Pyridoxal phosphate-6-azopheyl-2′,4′-disulphonic acid
SOM	Somatostatin
TTX	Tetrodotoxin
UTP	Uridine 5′-triphosphate

UDP Uridine 5′-diphosphate
VIP Vasoactive intestinal peptide

Introduction

The enteric nervous system (ENS) is located in the wall of the gut and is composed of two ganglionated plexes: the myenteric plexus and submucosal plexus. The ENS regulates and coordinates the activity of the gastrointestinal (GI) tract. The neurons of the ENS are specialized for sensory, motor, and interneuronal roles and together form multiple complex circuits for driving reflexes [1]. Enteric neurons receive some synaptic inputs from the sympathetic and the parasympathetic nervous systems but receive the majority of their inputs from other enteric neurons within the ENS. Synaptic transmission in the ENS utilizes fast ligand-gated ion channels and slow G-protein-coupled receptors to transmit and process information [2, 3]. Purinergic receptors including P2X, P2Y, and adenosine receptors have been localized to neurons in the ENS. A primary role for P2X receptors has been found in mediating fast synaptic transmission while P2Y receptors predominately mediate slow synaptic transmission and adenosine receptors mediate presynaptic inhibition. Together, these receptors affect many enteric reflexes and motor patterns [see review in Chapter 2 of this issue, 4].

In the present article, we review the evidence for P1 and P2 receptors in the ENS and examine the role they play in mediating fast and slow synaptic transmission and in presynaptic inhibition of transmission. We will also look at the molecular properties of the native receptors, their signaling mechanisms and their role in gastrointestinal pathology. The reader is directed to other chapters in this issue for more detailed information on the role purinergic transmission plays in reflexes, and on the role of adenosine in secretion [4, 5].

Enteric neurons

The enteric neurons can be classified by their shape, projection pattern, electrophysiology, and their neurotransmitter or chemical content [6–9]. The neurons that use ATP or adenosine as transmitters are termed purinergic neurons. The following summarizes the major subtypes and electrophysiological properties of enteric neurons (Fig. 1), highlighting those neurons that are purinergic.

Enteric intrinsic primary afferent neurons

There are enteric neurons that are sensitive to chemical or mechanical cues from the gastrointestinal tract with cell bodies in the myenteric and submucous plexes. They are properly referred to as intrinsic primary afferent neurons (IPANs) and are separate from the extrinsic primary afferent neurons whose cell bodies are in dorsal root or nodose ganglia [10]. In this review we will simply refer to them as sensory neurons. Many enteric sensory neurons have "AH"-type electrophysiological characteristics which are defined as having an action potential afterhyperpolarization lasting >1 s [11–13]. Most sensory neurons have smooth cell bodies and are multipolar with projections that run to the mucosal epithelium, between the myenteric plexus and the submucosal plexus, and to their own and nearby ganglia [14]. They form synapses with all other neuronal types. To date there is no evidence that enteric sensory neurons release ATP as a neurotransmitter.

Interneurons

Enteric interneurons are primarily found in the myenteric plexus where there is generally one subtype of interneuron (two types in proximal colon) with an ascending projection and at least three subtypes of interneuron with a descending projection. There are also a small number of intestinofugal neurons that send a projection to prevertebral ganglia [1]. All interneurons have a single axon, but there is a mix of dendritic morphology. Interneurons are characterized electrophysiologically as "S" neurons, which are those neurons

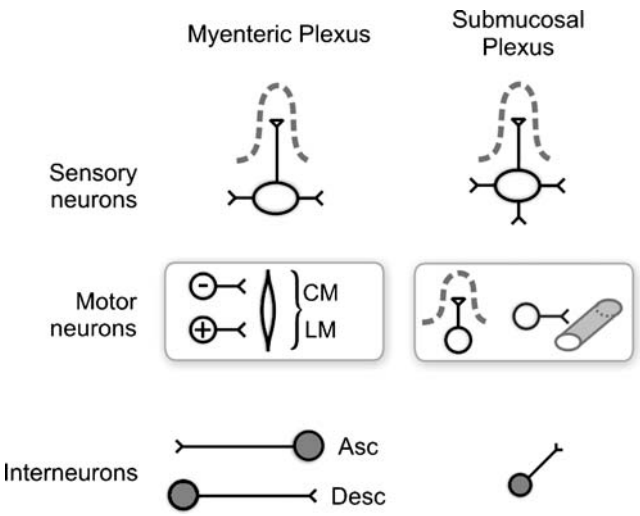

Fig. 1 Diagram showing the major functional subtypes of neuron in the enteric nervous system. The major functional types are listed *on the left* and the two plexes, the myenteric and the submucosal, are listed *at the top*. Enteric sensory neurons are found in both the myenteric and submucosal plexes. The inhibitory (−) and excitatory (+) motor neurons to the circular (*CM*) and longitudinal (*LM*) smooth muscle are only found in the myenteric plexus while secretomotor neurons and vasodilator neurons are mainly found in the submucosal plexus. Ascending (*Asc*) and descending (*Desc*) interneurons are found in the myenteric plexus, while in the submucosal plexus, only a small population of local interneurons is found

in which a single stimulus to an interganglionic nerve bundle can trigger a fast EPSP [11, 12]. They can differ in their level of excitability and in their ability to fire action potentials repetitively [12, 15, 16]. In the myenteric plexus, ATP is utilized as a transmitter or co-transmitter by at least one type of descending interneuron during local reflexes [4, 17]. These descending interneurons are immunoreactive for nitric oxide synthase (NOS) and vasoactive intestinal polypeptide (VIP) and have Dogiel type I dendrites. In the submucous plexus, interneurons comprise <15% of the population of neurons; however, they do not appear to release ATP or adenosine as a neurotransmitter.

Motor neurons

Enteric motor neurons are found in the myenteric and submucous plexes. They have a single axon and short dendrites with Dogiel type I morphology. In the myenteric plexus, there are four functional classes of motor neuron to the smooth muscle layers of the intestine [1, 9]: inhibitory or excitatory motor neurons to either the circular or longitudinal smooth muscle layers. All motor neurons have S-type properties and, like the interneurons, they can differ in their level of excitability [12]. In the myenteric plexus, inhibitory motor neurons to the circular muscle are immunoreactive for NOS and VIP and use ATP as a co-neurotransmitter [1, 6, 18]. In the submucous plexus, there are two types of excitatory secretomotor neurons going to the epithelium and at least one type of vasodilator neuron to the submucous arterioles. Noncholinergic secretomotor/vasodilator neurons are immunoreactive for dynorphin, galanin, and VIP. These neurons account for ~45% of submucosal neurons, and they may utilize ATP as a co-neurotransmitter [19, 20].

Purinergic receptors

Purinergic receptors include P1 and P2 receptors that are activated by the endogenous purines, adenosine and ATP, respectively. Thus, transmission involving either receptor is called purinergic [21]. Adenosine and ATP are recognized as either neurotransmitters or neuromodulators in the central nervous system [e.g., 22], the peripheral nervous system [e.g., 23, 24] and the ENS [2, 25].

Adenosine acts through G-protein-coupled P1 receptors that are commonly referred to as adenosine receptors (A_1, A_2, etc). ATP acts at both ligand-gated P2X receptors/ion channels [26, 27] and at G-protein-coupled P2Y receptors [28, 29]. Adenosine is thought to be liberated from cells as a consequence of metabolic stress or as a breakdown product of released ATP. In contrast, ATP is thought to be primarily released from neuronal sources as a neurotransmitter, although there is evidence of ATP release from nonneuronal cells such as working muscle [29].

P1 (adenosine) receptors

P1 receptors are typical G-protein-coupled receptors that act through modulation of adenylyl cyclase [21]. At least four genes for adenosine receptor subtypes have been cloned. These genes encode for A_1, A_{2A}, A_{2B}, and A_3 receptors. Adenosine acts as an agonist at all of these receptors with AMP and ADP showing progressively weaker interactions. ATP is not, by definition, considered an agonist at these receptors. The pharmacology of the adenosine receptors is characterized mainly by selective receptor antagonists. See Ralevic and Burnstock [21] for a full review of the P1 adenosine receptors.

P2 (ATP) receptors

P2X receptors are nonselective cation channels that open upon binding a ligand. The molecular structure of a P2X receptor is probably a trimer consisting of one or more different subunits [30]. There are seven subtypes of P2X receptor found in adult mammalian tissue, $P2X_{1–7}$ [27, 31]. Each subunit has two membrane-spanning domains (TM1 and TM2) and a large extracellular domain [26]. TM1 is responsible for channel gating, and TM2 forms the ion pore. The extracellular loop is suggested to be involved in binding of two molecules of ATP. Studies of heterogeneous expression of the P2X-receptor subunits have generated a series of data for the properties of different P2X subunit combinations [27]. P2X receptors can be composed of the same or different subunits to form homomers or heteromers, respectively. All P2X subunits can form homomers, except the $P2X_6$, which only combines with other subunits. All P2X subunits can also form heteromers, except the $P2X_7$ subunit, which can only form a homomer. The specific composition of the P2X subunits determines the unique pharmacological and physiological properties of the native receptors. For example, ATP is a ligand at all P2X receptors, but the ATP-analogue α,β-methylene-ATP is a more selective agonist at $P2X_1$ and $P2X_3$ subunit containing receptors than at other subtypes, while 2-methylthio-ATP is a more selective agonist at $P2X_2$ and $P2X_3$ receptors [21].

P2Y receptors are G-protein-coupled (metabotropic) receptors [29]. In mammals, there are at least eight genes encoding subtypes of P2Y receptor with more likely awaiting discovery [28, 32]. The five main receptor subtypes are the $P2Y_{1,2,4,6,11}$. All couple to activation of phospholipase C and, in addition, $P2Y_{11}$ couples positively to adenylyl cyclase. ATP is a ligand at all of these receptors, but UTP, UDP, or ADP may be more potent and can be used pharmacologically to distinguish between receptor

subtypes [28]. Similarly, α,β-methylene-ATP is not an agonist at P2Y receptors, but 2-methylthio-ATP is an agonist at $P2Y_1$ and $P2Y_{11}$ receptors [21].

Adenosine and P1 receptors in the ENS

The production of adenosine

Many studies have shown that P_1 receptors are activated by endogenous adenosine, but whether adenosine is stored in synaptic vesicles and released as a neurotransmitter is not clear. One way adenosine may be made available is from the breakdown of released ATP. ATP is degraded by extracellular enzymes known as ectonucleotidases of which there are several classes [33]. For example, the production of adenosine from the hydrolysis of ATP released from activated smooth muscle cells has been demonstrated [34]. Adenosine may then act at its own receptors before itself being broken down to inosine by the enzyme adenosine deaminase. Studies in the ENS have failed to show an appreciable effect when ATP is applied and P1 receptors are blocked [35, 36] or when breakdown of ATP is prevented [37], suggesting that this route for adenosine formation, although plausible, may not be physiologically relevant.

Another route by which adenosine may be released into the extracellular space is a nonspecific release from normal cells including neurons. Electrically evoked adenosine release has been demonstrated from myenteric plexus-longitudinal muscle preparations [38]. Myenteric neurons were the main source of endogenous adenosine, since blockade of action potentials with tetrodotoxin (1 μM) or omission of Ca^{2+} (plus EGTA, 1 mM) in the solution essentially abolished nucleoside release, while adenosine outflow remained unchanged when smooth muscle contractions were prevented by nifedipine [37]. Adenosine can be released under basal conditions, but its release is often increased under metabolically stressful conditions such as ischemia, inflammation, or cell damage [e.g., 39]. In the gut, endogenous concentrations of adenosine have been found to vary with the pO_2 in the myenteric plexus [40].

Identification and expression of P1 subtypes in the ENS

Adenosine applied to the intestine can influence intestinal functions by directly acting at adenosine receptors on smooth muscle [41–43] or by indirectly acting at adenosine receptors in the ENS to regulate neurotransmitter release [42, 44, 45]. This evidence suggests that adenosine receptors are located within the GI tract and, thus, that endogenous adenosine may play a physiological role. For example, regulation of secretion by adenosine has been established [see Chapter 3, this issue, 5]. On the other hand, homozygous A_1 adenosine receptor–null mice are viable and without gross behavioral or anatomic abnormalities [46], suggesting other receptors can compensate for the loss of the A_1 receptor.

Adenosine may mediate relaxation of the intestine through two different inhibitory receptor subtypes. In the guinea-pig distal colon, A_1 receptors are on enteric neurons and A_{2B} receptors are on the smooth muscle [41, 47]. In contrast, in murine proximal colon, A_1 receptors have been identified on smooth muscle and mediate relaxation, while in distal colon, there are additionally A_{2B} receptors localized on myenteric inhibitory motor neurons releasing NO [42]. In rat small intestine, the ascending and descending reflexes in the myenteric plexus are modulated by release of endogenous adenosine acting at A_1 receptors [48]. More recently, the coexistence of both inhibitory A_1 and facilitatory A_2 adenosine receptors in the rat myenteric plexus has been demonstrated [49]. Finally, in the human enteric nervous system, the distribution of adenosine receptor mRNA and protein has been demonstrated [50]. The A_1 receptor is expressed in jejunal myenteric neurons and colonic submucosal neurons, while in the jejunal myenteric plexus, A_{2B} receptor immunoreactivity was found in more neurons than was the A_{2A} receptor [50] [see Chapter 3, this issue, 5].

Postsynaptic P1 receptors may not mediate synaptic potentials

In the ENS, there is no clear evidence that synaptic potentials are caused by the release of adenosine acting at postsynaptic receptors. This is despite very good evidence that exogenously applied adenosine can cause a closure of potassium channels and a slow EPSP-like depolarization in some AH neurons [51]. These effects are thought to be through the A_{2A} receptor, which couples positively with adenylyl cyclase and causes accumulation of cAMP. This is supported by the finding that exogenous AMP also acts at A_{2A} receptors to cause slow EPSP-like depolarizations [52]. Exogenous application of adenosine also causes a slow hyperpolarization in some AH neurons [53] through the A_1 receptor and inhibition of adenylyl cyclase [51, 54]. Inhibition of adenylyl cyclase reduces the depolarization produced by many neuropeptides that presumably work by increasing cAMP [53, 55]. Nonetheless, there is no evidence for a slow IPSP mediated by endogenously released adenosine.

Presynaptic P1 receptors inhibit transmitter release and synaptic transmission

The most important function of adenosine in the ENS seems to be that of a modulatory substance that is present

tonically and changes only slowly. Experiments have clearly shown that endogenous adenosine is present and can act on its own receptors [for review, see 25]. Activation of A_1 receptors can cause inhibition of contraction in a TTX-insensitive manner in murine proximal and distal colon [42]. Antagonists at A_1 receptors can enhance the release of acetylcholine or tachykinins from myenteric ganglia [44, 56, 57]. This modulation is likely to be through A_1 receptors that are presynaptic. Similarly, fast EPSPs are reduced in the presence of adenosine (Fig. 2a) or AMP, an effect blocked by A_1 receptor antagonists [51, 52, 54]. The mechanism underlying presynaptic A_1 receptor inhibition of transmitter release involves an inhibition of adenylyl cyclase or phospholipase C with one result being an increase in potassium conductance and/or a decrease in calcium conductance [58]. Although presynaptic inhibition of transmitter release appears to be the main role of P1 adenosine receptors, there is one report that P2 ATP receptors may participate in presynaptic inhibition [36].

ATP and P2 receptors in the ENS

The source of ATP in the ENS

Localization of ATP is more difficult than localization of other substances [21]. Unlike classical transmitters or neuropeptides, purines are located throughout a cell making standard histochemical approaches nonspecific. Quinacrine staining has been used by some groups to visualize the high concentrations of ATP in vesicles [e.g. 59], although the specificity of this technique has not been well-established. In the ENS, quinacrine localization does not demonstrate unequivocally that there are ATP-containing nerve terminals or cell bodies; thus, the functional classes of neuron that might release ATP cannot be found directly.

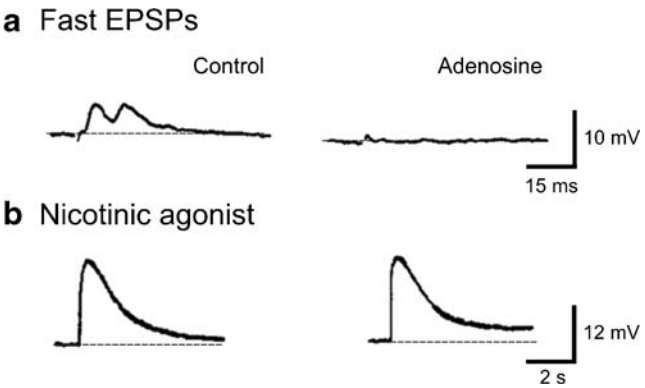

Fig. 2a, b Presynaptic inhibition of fast synaptic transmission in an S neuron in the submucous plexus of guinea pig ileum. **a** *Left* A fast EPSP was evoked by a single stimulus to an interganglionic fibre tract. *Right* Exposure to adenosine for 5 min suppressed the fast EPSP. **b** *Left* Depolarization to pressure application of DMPP (1 μM). *Right* During blockade of the fast EPSP, the depolarization to DMPP was unchanged in the presence of adenosine. Adapted from [114]. Copyright © Gastroenterology

Studies from vagal and sympathetic nerves have suggested that ATP is a co-transmitter with ACh, NE or histamine [60, 61]. In vivo, ATP released from these sources may reach and act on enteric purinergic receptors [20]. In the myenteric plexus, ATP can be released from varicosities by nicotinic-receptor agonists [62, 63]. Studies on the enteric circuitry point toward ATP being co-released from noncholinergic neurons using VIP or NO as co-transmitters [64]. This is supported by indirect evidence from the guinea pig colon that suggests ACh and ATP are not co-stored, as there is an increase in synaptic ATP release but not ACh release from inflamed tissue [65]. For more information on the role of ATP in the enteric circuitry see Chapter 2, this issue [4].

P2X receptors mediate fast EPSPs in the ENS

Fast EPSPs in enteric S neurons are short (~30 ms), high amplitude (>10 mV) depolarizations. A single fast EPSP can be triggered by a single presynaptic stimulus and can initiate an action potential. Fast EPSPs are the major form of communication between enteric neurons and are mainly mediated by ACh acting through nicotinic receptors. Data in the last 10 years have, however, suggested that a significant proportion of fast synaptic transmission is purinergic and that this component may be important in disease states.

Properties of P2X receptors in the ENS

Electrophysiological characterization of P2X receptors has been made in cultured guinea-pig myenteric neurons and in intact LMMP preparations. ATP-induced whole-cell currents show inward rectification. This is due to a decrease in the open probability of single channels at more positive membrane potentials as a single-channel current-voltage relationship is linear [66]. A reversal potential of 0 mV indicates that P2X receptors have a nonselective permeability to cations. Inward currents induced by ATP acting at myenteric P2X receptors desensitize by 80% in 7 s [66]. In guinea-pig myenteric plexus, P2X receptors have also been shown to couple to a calcium-dependent conductance that mediates an afterhyperpolarization in some NOS-positive S neurons [67].

Interactions between P2X receptors and other ligand-gated ion channels

Studies have shown that there is a functional interaction between P2X and nicotinic acetylcholine receptors [68–70]. P2X receptors are co-expressed in at least 67% of

myenteric neurons with nicotinic acetylcholine receptors. Simultaneous activation of nicotinic and P2X receptors produces a response that is smaller in amplitude than the predicted sum of responses caused by individual activation of each receptor. Recently, $GABA_A$ currents or current produced by activation of 5-HT_3 receptors has also been found to occlude P2X currents in much the same way [71, 72]. Taken together, these data suggest that a functional interaction between P2X and other ligand-gated channels may be the norm and thus physiologically important.

Localization of P2X receptors in the ENS

In the guinea-pig ileum, $P2X_2$ receptors have been localized in the myenteric plexus to populations of NOS-positive interneurons or motor neurons, and on intrinsic sensory neurons [73]. Ninety percent of the intrinsic sensory neurons stained for the $P2X_2$ receptor, with one-third of these staining strongly and the rest staining weakly. In the mouse intestine, $P2X_2$ receptor mRNA has been localized to a much smaller population of myenteric neurons of unknown functional class [74]. In the guinea pig, $P2X_3$ receptors are found on a variety of myenteric neurons, including some NOS-positive neurons (inhibitory motor and descending interneurons) and ascending interneurons or longitudinal muscle motor neurons, but not on sensory neurons [75, 76]. Similar data in mice have shown the presence of $P2X_2$, $P2X_3$, and $P2X_5$ receptors on many myenteric and submucosal neurons [77] although only $P2X_2$ receptors have subsequently been found to participate in fast synaptic transmission [78] (see below).

In the rat, Xiang and Burnstock [79] found mRNA and immunoreactivity for $P2X_2$ and $P2X_3$ receptors throughout the GI tract, from the stomach to the colon. In the myenteric plexus, 20% of $P2X_2$ receptors and 80% of $P2X_3$ receptors are localized to intrinsic sensory neurons. In the submucous plexus, intrinsic sensory neurons comprised 20% of the $P2X_2$-receptor-positive neurons and 40% of the $P2X_3$-receptor-positive neurons [79]. Recent data from Van Crombruggen et al. have supported this by showing that $P2X_2$ and $P2X_3$ receptors are found on nerve fibers in the myenteric and submucous plexus of rat distal colon [80].

Fast synaptic transmission

Interneurons and motor neurons (S neurons) In guinea-pig ileum, electrophysiological recordings have indicated that fast EPSPs from 67% of myenteric S neurons are sensitive to the P2X-receptor antagonists PPADS or suramin [2, 81] (Fig. 3a). The purinergic fast EPSPs are not, however, evenly distributed along the GI tract. Recordings from different segments of the gut indicate that purinergic fast EPSPs are most common in the ileum as compared with the duodenum, jejunum, taenia coli, proximal and distal colon, but are absent in gastric corpus [82, 83]. Evidence from combined electrophysiological and lesion studies have shown that some of these P2X receptors are in descending pathways [84]. These data have been extended by recent studies in the submucosal plexus of the guinea pig where P2X-mediated fast EPSPs have been identified [19] (Fig. 3b). Together these data suggest that endogenous ATP acting at P2X receptors mediates fast EPSPs between many neurons in the ENS.

Intrinsic sensory neurons (AH neurons) P2X receptors may also mediate activation of AH neurons and contribute to initiation of peristalsis [85]. ATP applied to the cell bodies evoked a large depolarization in most myenteric AH neurons that was blocked by PPADS and, unexpectedly, potentiated by suramin. Similarly, ATP applied to small areas of the mucosal epithelium activated the mucosal sensory nerve terminals of the AH neurons. PPADS blocked the activation of the nerve terminals and a 5-HT_3-receptor antagonist reduced it. Thus, ATP may act directly through P2X receptors on the sensory nerve terminal and through the release of serotonin from enterochromaffin cells. These data suggest that ATP participates in both the sensory transduction of stimuli from the gut lumen and in the subsequent initiation and propagation of enteric reflexes.

Although $P2X_2$ receptors have been localized to AH neurons [78, 86], fast excitatory synaptic inputs are rarely recorded from these neurons in guinea pig [86] or mouse [78, 87]. Fast EPSPs that have been recorded from sensory neurons are much smaller in amplitude than in S neurons [12, 88]. Fast EPSPs in sensory neurons appear to be mediated by ACh acting at nicotinic receptors, but whether purines also participate is not yet clear.

Subtypes of P2X receptor One question remaining is what subtypes of P2X receptor are responsible for fast synaptic transmission in S neurons and depolarization in AH neurons. Immunohistochemical studies have localized $P2X_2$ and $P2X_3$ subunits to some myenteric neurons in guinea pig [73, 75, 76]. Recent studies have utilized mice deficient in $P2X_2$ or $P2X_3$ receptors to investigate these subtypes in S and AH neurons [78, 89]. S neurons in tissues from $P2X_2^{+/+}$ mice were depolarized by ATP but not by α,β-mATP. This result suggests that S neurons express $P2X_2$ homomeric receptors as α,β-mATP does not activate $P2X_2$ homomers, but does activate $P2X_3$ homomeric and $P2X_{2/3}$ heteromeric receptors [27, 90]. ATP failed to elicit a depolarization in S neurons from $P2X_2^{-/-}$ mice, and fast EPSPs were not reduced by PPADS, a nonspecific P2-receptor antagonist (Fig. 4a). Fast EPSPs from $P2X_3^{-/-}$ mice were reduced by PPADS to a similar level as were fast

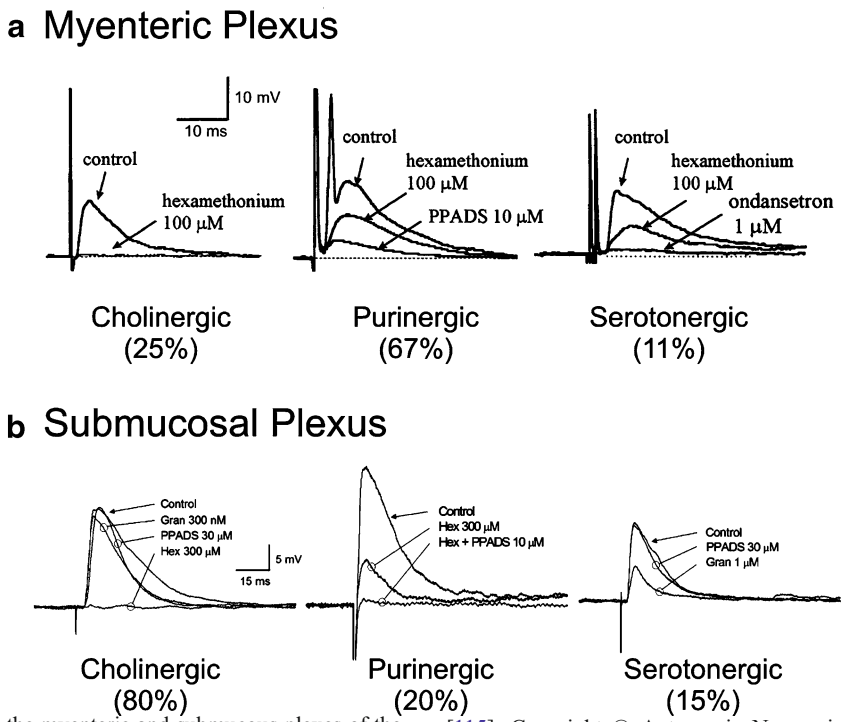

Fig. 3a, b Fast EPSPs in the myenteric and submucous plexes of the guinea pig ileum have a prominent purinergic component. **a** Pharmacologically distinct fast EPSPs from myenteric neurons. *Left* A fast EPSP that was blocked by the nicotinic-receptor antagonist hexamethonium (100 μM). *Middle* A fast EPSP that is partly reduced by hexamethonium and the rest is blocked by PPADS, an antagonist that blocks P2 receptors. *Right* A fast EPSP that is partly reduced by hexamethonium and is completely inhibited by the subsequent addition of the 5-HT-receptor antagonist ondansetron. Adapted from [115]. Copyright © Autonomic Neuroscience. **b** Pharmacologically distinct fast EPSPs from submucosal neurons. *Left* Application of the nicotinic-receptor antagonist hexamethonium (300 μM, Hex) abolished this fast EPSP. *Middle* In this neuron hexamethonium depressed the fast EPSP, and PPADS (10 μM) abolished the remainder. *Right* PPADS (10 μM) had no effect on this fast EPSP, but granisetron depressed it by approximately 50%. Adapted from [19]. Copyright © Journal of Physiology

EPSPs from $P2X_3^{+/+}$ mice (Fig. 4b). Based on these results, it was concluded that the P2X receptor mediating fast EPSPs in murine S neurons is a $P2X_2$ homomeric receptor.

In a parallel study in the guinea pig ileum, all S neurons that were sensitive to ATP were depolarized by α,β-mATP [67] but only 17% of AH neurons were sensitive to α, β-mATP (J. Ren, personal observation). Previous pharmacological studies have found that α,β-mATP-sensitive P2X-receptor subtypes ($P2X_1$ or $P2X_3$) contribute to fast EPSPs [82]. TNP-ATP, a selective antagonist for $P2X_1$ or $P2X_3$ receptors, reduced ATP-induced depolarization and reduced fast EPSP amplitude in S neurons. These results are not, however, consistent with guinea pig myenteric neurons maintained in primary culture, which do not desensitize to α,β-mATP [66]. One important difference between these studies is that the cultured enteric neurons were isolated from new-born guinea pigs while the intact preparations were dissected from adults. Thus, the differences seen could be caused by a switch from one P2X-receptor subtype to another during development. In support of this idea it has been shown in the rat stomach that the levels of $P2X_3$ receptor expression undergo developmental changes [91].

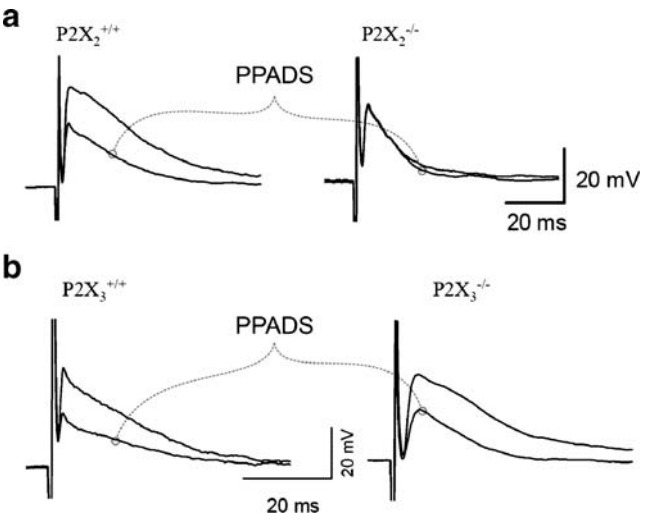

Fig. 4a, b Fast EPSPs in myenteric S neurons from mice deficient in $P2X_2$ (A.) or $P2X_3$ (B.) receptors. **a** Recordings from S neurons in $P2X_2^{+/+}$ (*left*) and $P2X_2^{-/-}$ (*right*) mice. The fast EPSPs recorded from neurons in tissues from $P2X_2^{+/+}$ mice were inhibited by PPADS (10 μM) while those from neurons in $P2X_2^{-/-}$ tissues were unaffected (*right*). **b** Fast EPSPs recorded from S neurons in $P2X_3^{+/+}$ (*left*) and $P2X_3^{-/-}$ (*right*) mice; both were inhibited by PPADS (10 μM). Adapted from [78, 89]. Copyright © Journal of Physiology

Together, these data suggest that in guinea pig myenteric S neurons, fast EPSPs are mediated by $P2X_3$ subunit-containing receptors while AH neurons probably express $P2X_2$ homomers. In contrast, mouse myenteric S neurons express $P2X_2$ homomers that mediate fast synaptic transmission, and AH neurons express $P2X_3$ subunit-containing receptors.

ATP is also a transmitter in descending excitatory reflexes [92, 93] as this pathway is sensitive to P2X receptor antagonists. P2X receptor-mediated fast synaptic transmission participates in descending inhibitory reflex pathways in guinea pig small intestine [17] but not in rat small intestine or guinea pig colon [94, 95] [see Chapter 2, this issue, 4]. Despite this role for P2X receptors, neither the phenotype of the $P2X_2$ nor $P2X_3$ knockout mice shows any outstanding abnormality [78, 89]. The mechanisms that maintain normal GI physiology when purinergic receptors are lost remains unclear.

P2Y receptors mediate slow EPSPs in the ENS

Slow EPSPs in the ENS are long-lasting (>1 s), moderate amplitude (~5 mV) depolarizations that generally require a train of presynaptic stimuli to be evoked. The excitability of the neuron is greatly enhanced by a slow EPSP, often leading to repetitive firing of action potentials. Slow synaptic transmission within the ENS is mainly mediated by tachykinins acting at neurokinin receptors and ACh acting at muscarinic receptors. There are, however, a host of other candidates for mediators of slow EPSPs, including ATP acting at P2Y receptors.

Localization of P2Y receptors in the ENS

Work in the mouse myenteric plexus has shown that immunoreactivity for $P2Y_1$ receptors is located on NOS immunoreactive neurons (descending interneurons or inhibitory motor neurons) and that mRNA can be found in both myenteric and submucous plexes [74]. Work in the submucous plexus has suggested that neurons expressing $P2Y_1$ receptors are secretomotor [20, 96]. More recently, the $P2Y_1$ receptor has been cloned from the guinea pig submucous plexus and characterized in human embryonic kidney cells [97], though the original neuronal class expressing the receptor was not identified. $P2Y_2$ receptors have been localized to the rat colon myenteric plexus where almost a quarter of these neurons co-expressed nNOS [80]. $P2Y_2$ receptor immunoreactivity was also co-localized with a majority of calretinin immunoreactive neurons in the myenteric plexus (ascending interneurons and excitatory motor neurons) and all the NPY immunoreactive neurons in the submucous plexus (secretomotor neurons) [98]. Immunoreactivity for $P2Y_6$ receptors was found in all regions of the guinea pig gastrointestinal tract in the myenteric plexus, but not the submucous plexus [99]. About 32% of $P2Y_6$ receptor immunoreactivity was co-localized to NOS immu-

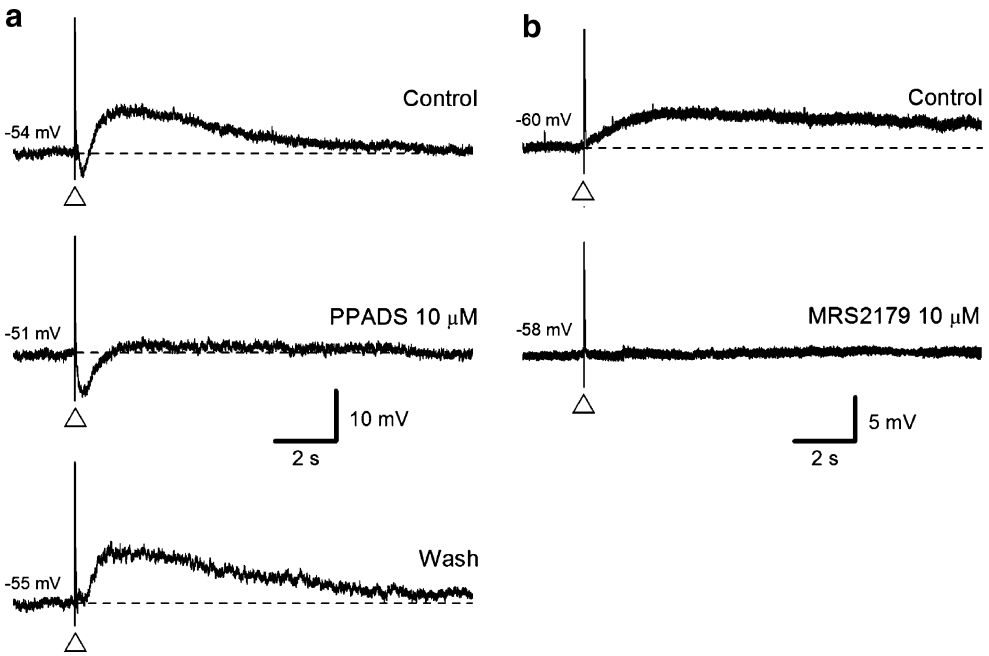

Fig. 5a, b Slow EPSPs in the submucous plexus of guinea pig ileum are blocked by P2Y receptor antagonists. Voltage traces taken from two submucosal neurons. **a** A single-pulse electrical stimulus evoked, in the following order: a fast EPSP, an intermediate EPSP, a small IPSP and a slow EPSP. Application of the P2-receptor antagonist PPADS (30 μM, *middle trace*) abolished the slow EPSP. Note, the IPSP amplitude is enhanced in the *middle trace* by the blockade of the intermediate EPSP and the slow EPSP. **b** The selective $P2Y_1$-receptor antagonist MRS 2179 (10 μM) abolished the slow EPSP while the fast EPSP was spared. The IPSP in this cell had already been blocked with idazoxan. Adapted from [19]. Copyright © Journal of Physiology

noreactive neurons (descending interneurons and inhibitory motor neurons) and about 45% to calretinin immunoreactive neurons (ascending interneurons and excitatory motor neurons). In the guinea pig small intestine, the $P2Y_{12}$ receptor has been localized to calbindin immunoreactive neurons (AH/sensory neurons) [99], while in the rat colon, $P2Y_{12}$ receptors have been localized to the myenteric plexus [80].

Properties of P2Y receptors in the ENS

Christofi et al. [35] looked at cultured myenteric neurons from guinea pig and showed that there is a rise in intracellular calcium associated with application of ATP and activation of P2 receptors. This was confirmed in submucosal neurons where a fast P2X receptor-mediated and a slow P2Y receptor-mediated calcium rise and depolarization were identified [100]. In contrast, a rise in calcium in the intrinsic sensory neurons can activate the potassium conductance underlying the AHP. In submucous neurons, the $P2Y_1$ receptor has been linked to mobilization of Ca^{2+} from intracellular stores by activation of phospholipase C and synthesis of inositol 1,4,5-trisphosphate with the subsequent depolarization associated with a conductance increase [20].

Slow synaptic transmission

In the guinea pig ileum myenteric plexus, electrophysiological recordings have shown that P2Y receptors are present on S neurons (interneurons and motor neurons). When exogenous ATP was applied, there was a profound depolarization of the S neurons [101]. Recent work in the myenteric plexus has found that S neurons have distension-evoked slow EPSPs that are blocked by PPADS [102]. These slow EPSPs only occurred in NOS-positive descending interneurons [103]. These data are supported by work in the mouse demonstrating that $P2Y_1$ receptors are on NOS-positive myenteric neurons [74].

Recent studies in the submucosal plexus of the guinea pig have demonstrated P2Y-mediated slow EPSPs (Fig. 5) that are blocked by the selective $P2Y_1$ antagonist MRS 2179 [19, 104, 105]. Interestingly, a single-stimulus-evoked EPSP with a time course between that of a fast and a slow EPSP (an intermediate EPSP) was shown to be blocked by PPADS, suramin, and the selective $P2Y_1$ receptor antagonist MRS 2179 [19]. Most submucous neurons, including most non-cholinergic secretomotor neurons (VIP-IR) exhibited an intermediate EPSP that could occur spontaneously and was sometimes large enough to initiate action potentials.

Electrophysiological studies in guinea pig ileum have also shown P2Y receptors on the intrinsic sensory neurons of the myenteric plexus. Here they appear to mediate a hyperpolarization of the membrane due to an opening of the calcium-activated potassium conductance [85, 101]. This has been supported by calcium imaging studies that suggest a P2Y receptor is coupled to release of internal calcium [35] possibly through a $P2Y_1$ receptor [20].

Intestinal pathologies and purinergic signaling

Changes in purinergic signaling in the ENS may contribute to some pathological mechanisms in the GI tract. Recent evidence points toward an alteration in purinergic synaptic transmission in inflamed tissue. Evoked fast EPSPs in myenteric neurons increase in amplitude following trinitrobenzene sulphonic acid (TNBS) colitis [106]. Later electrophysiological studies of submucosal neurons in guinea pig colon have shown the increase in fast EPSP amplitude could be attributed to a P2X component in inflamed tissue [65]. This fits well with earlier work on inflammatory bowel patients that showed an increase in $P2X_3$ immunoreactivity in the colonic enteric neurons [107]. One question that remains is what mechanism underlies the increase in purinergic fast EPSPs in inflamed tissue? Upregulation of presynaptic ATP release and/or alteration of postsynaptic P2X receptors appear to contribute in the submucous and myenteric plexes, respectively [65, 106]. Recently this work has been extended to show that, following resolution of colitis, the increase in purinergic fast EPSP amplitude remains [108] and that there is an increase in fast EPSP amplitude in noninvolved tissue remote from the inflammation [109].

Changes in adenosine receptors have also been found in a rabbit model of chronic ileitis where an up-regulation of A_1 and A_3 receptors at the transcriptional level was found [110]. Other studies have shown that stimulation of A_{2A} receptors can reduce inflammation in the intestinal mucosa in rabbits and mice [111] and reduce tissue injury and inflammation in mice with toxin A-induced enteritis [112]. A recent study utilized a high-density oligonucleotide microarray analysis to study TNBS-induced colitis [113]. It was found that receptors for $P2X_{1,4,7}$ and $P2Y_{2,6}$ and all adenosine receptors were upregulated while there was a down regulation of $P2X_2$ and $P2Y_{1,4}$ receptors. Activation of A_3 receptors reversed many, but not all, of the changes in gene expression due to inflammation [113].

Summary and conclusions

In the enteric nervous system, ATP plays a role as a neurotransmitter between enteric neurons while adenosine probably plays a role as a neuromodulator. The regulated release of ATP from enteric neurons has been shown to

mediate fast synaptic potentials via $P2X_2$ and $P2X_3$ receptors and slow synaptic potentials via $P2Y_1$ receptors. The role of adenosine is as a modulator of presynaptic inhibition of transmission and acts mainly via A_1 receptors to inhibit gastrointestinal activity. Finally, both adenosine and ATP receptors have been shown to be altered during gastrointestinal pathologies. These conclusions are drawn mainly from guinea pig or rodent models, which have revealed important species differences.

A challenge for future studies will be to determine if these conclusions hold true for all mammalian species including humans. This is important as drugs targeted at purine receptors have shown promise for ameliorating some of the changes seen during gastrointestinal diseases.

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