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Publication details:
Purinergic Signalling
v. 4
Chapter No. 3
pp. 255-266
1573-9538 (ISSN)

Publication Date:
2008

Publisher DOI:
http://dx.doi.org/10.1007/s11302-007-9088-5

Review

Purinergic receptors and synaptic transmission in enteric neurons

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Key words: adenosine receptors; enteric nervous system; gastrointestinal tract; P2X receptors; P2Y receptors; purines; synaptic transmission.

Running head: Purinergic synaptic transmission in the ENS

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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, the 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, the 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.

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 examine the role they play in mediating fast and slow synaptic transmission and in presynaptic inhibition of transmission. Finally, we will focus on the molecular properties of the native receptors, their signaling mechanisms and their role in gastrointestinal pathology.

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. Finally, we will focus on 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 adenosine and secretion [4, 5].

The enteric nervous system (ENS)

The ENS contains different types of enteric neurons that vary in their shape, projection pattern, electrophysiological characteristics and their neurotransmitter content. Over the past 30 years, the enteric neurons have been categorized by these characteristics into 18 different classes [6-9]. The three major classes of neuron in terms of their function within the enteric circuitry are sensory neurons, interneurons and motor neurons. These can further be classified by their electrophysiological properties as either AH type neurons or S type neurons. Sensory neurons generally have AH type electrophysiological properties which are characterized by an afterhyperpolarization lasting 1-10 seconds [10-12], though in pig the AHP is small [13]. Interneurons and motor neurons have S type properties where a single stimulus to an inter-ganglionic nerve bundle can trigger a fast EPSP [10, 11]. The following summarizes the major sub-types and electrophysiological properties of enteric neurons (FIGURE 1) and highlights those that might release ATP as a neurotransmitter and are thus, purinergic neurons.

Sensory neurons

Enteric sensory neurons are found in the myenteric and submucous plexes. They are also referred to as "intrinsic primary afferent neurons (IPANs)", to differentiate them from the extrinsic primary afferent neurons whose cell bodies are in dorsal root and nodose ganglia [14]. Still others refer to them by their original designation of 'AH neuron' [3, 10]. Most enteric sensory neurons have smooth cell bodies and are multi-polar with projections that run to the mucosal epithelium, between the myenteric plexus and the submucosal plexus and to their own and nearby ganglia [15]. They form synapses with other sensory neurons, interneurons and motor neurons. In the myenteric plexus, the projections are predominately in the circumferential directions but there is a small group that also have a long projection in the anal direction [16]. While there is no evidence to date that the sensory neurons release ATP as a transmitter, there is good evidence that ATP is not involved in some motor reflex pathways [e.g., 17] and [see Chapter 2, this issue, 4].

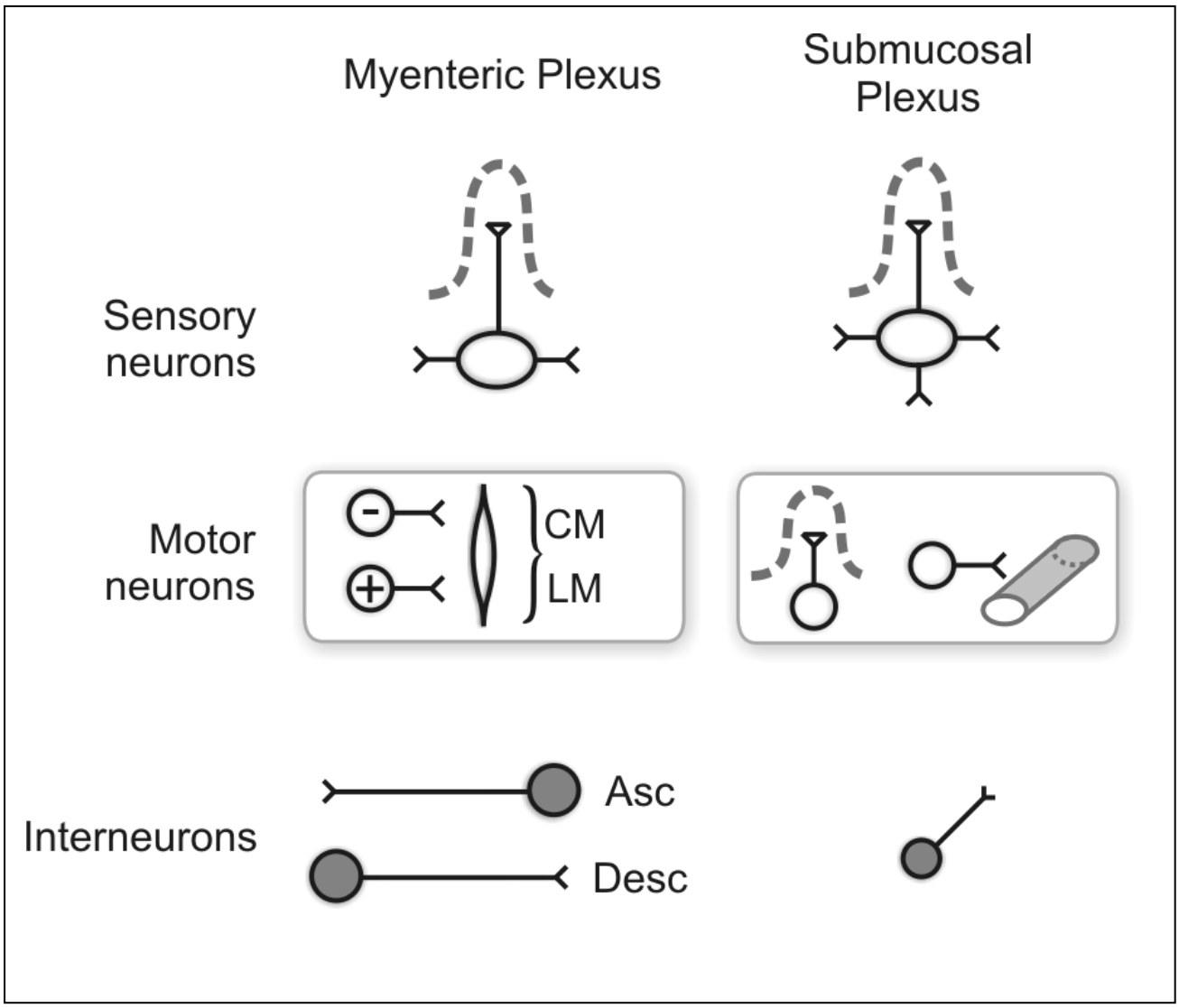

Figure 1. Diagram showing the major functional sub-types of neuron in the enteric nervous system. The major functional types are listed on the left column and the two plexes, the myenteric and the submucosal, are listed at the top. Sensory neurons are found in both the myenteric and submucosal plexes. 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.

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 ganglion [1]. All interneurons have a single axon but there is a mix of dendritic morphology. All interneurons also have S type properties but they can vary in their level of excitability and repetitive firing [11, 18, 19]. 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 neurons but 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]; either inhibitory or excitatory motor neurons to either the circular or longitudinal smooth

muscle layers. All motor neurons have S type properties but, like the interneurons, they can vary in their level of excitability [11]. In the myenteric plexus, inhibitory motor neurons to the circular muscle express NOS, VIP and use ATP as a co-neurotransmitter with NO and VIP [1, 6, 20]. In the submucous plexus, there are two types of excitatory secretomotor neurons going to the epithelium and at least one type of vasodilator neuron going to the submucous arterioles. Non-cholinergic secretomotor/vasodilator neurons are immunoreactive for dynorphin (DYN), galanin (GAL) and VIP. These neurons account for ~45% of submucosal neurons and they also utilize ATP as a neurotransmitter [21].

Purinergic receptors

Purinergic receptors include P1 and P2 receptors. They are activated by the endogenous purines, adenosine and ATP, respectively and, thus, transmission involving either is called purinergic [22]. Adenosine and ATP are recognized as either neurotransmitters or neuromodulators in the central nervous system [e.g., 23], the peripheral nervous system [e.g., 24, 25] and the ENS [2, 26].

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 [27, 28] and at G-protein coupled P2Y receptors [29, 30]. Adenosine is thought to be

liberated from cells as a consequence of metabolic stress or as a breakdown product of released ATP whereas ATP is primarily released from neuronal sources as a neurotransmitter. In addition, ATP has been found to be released from non-neuronal cells or from working muscle [30].

P1 (adenosine) receptors

P1 receptors are typical G-protein coupled receptors that act through modulation of adenylyl cyclase [22]. At least 4 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 & Burnstock [22] for a full review of the P1 adenosine receptors.

P2 (ATP) receptors

P2X receptors are non-selective cation channels which open upon binding a ligand. The molecular structure of a P2X receptor is probably a trimer consisting of one or more different subunits [31]. There are seven sub-types of P2X receptor found in adult tissue, $P2X_{1-7}$ [28, 32]. Each subunit has two membrane-spanning domains (TM1 and TM2) and a large

extracellular domain [27]. 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 [28]. 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. In contrast, all P2X subunits can 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-analog, 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 [22].

P2Y receptors are G-protein coupled (metabotropic) receptors [30]. There are at least eight genes encoding sub-types of P2Y receptor with more likely awaiting discovery [29, 33]. The five main sub-types of receptor 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 sub-types [29]. 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 [22].

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 [34]. For example, the production of adenosine from the hydrolysis of ATP released from activated smooth muscle cells has been demonstrated [35]. Adenosine may then act at its own receptors before itself being broken down to inosine by the enzyme adenosine deaminase. While plausible, studies in the ENS have failed to show an appreciable effect when ATP is applied and P1 receptors are blocked [36, 37] or when breakdown of ATP is prevented [38] suggesting that this route for adenosine formation may not be physiologically relevant.

Another route by which adenosine may be released into the extracellular space is a non-specific release from normal cells including neurons. Electrically-evoked adenosine release

has been demonstrated from myenteric plexus-longitudinal muscle preparations [39]. 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 [38]. 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., 40]. In the gut, endogenous concentrations of adenosine have been found to vary with the pO_2 in the myenteric plexus [41].

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 [42-44] or by indirectly acting at adenosine receptors in the ENS to regulate neurotransmitter release [43, 45, 46]. 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 [47] suggesting other receptors can compensate for the loss of the at least 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 on the enteric neuron and A_{2B} receptors are on the smooth muscle [42, 48]. 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 neurons releasing NO [43]. 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 [49]. More recently, the coexistence of both inhibitory A_1 and facilitatory A_2 adenosine receptors in the rat myenteric plexus has been demonstrated [50]. Finally, in the human enteric nervous system, the distribution of adenosine receptor mRNA and protein has been demonstrated [51]. The A_1 receptor is expressed in jejunal myenteric neurons and colonic submucosal neurons while in the myenteric plexus, A_{2B} receptor immunoreactivity was found in more neurons than was the A_{2A} receptor [51] and [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 slow EPSP-like depolarization in some AH

neurons [52]. These effects are thought to be through the A_{2A} receptor, which couples positively with adenylyl cyclase and causes accumulation of cAMP. This is support by the finding that exogenous AMP also acts at A_{2A} receptors to cause slow EPSP-like depolarizations [53]. Exogenous application of adenosine also causes a slow hyperpolarization in some AH neurons though the A_1 receptor and inhibition of adenylyl cyclase [52, 54], though again, 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 26]. Activation of A_1 receptors can cause inhibition of contraction in a TTX-insensitive manner in murine proximal and distal colon [43]. Antagonists at A_1 receptors can enhance the release of acetylcholine or tachykinins from myenteric ganglia [45, 55, 56]. This modulation is likely to be through A_1 receptors that are presynaptic. Similarly, fast EPSPs are reduced in the presence of adenosine (Figure 2A) or AMP, an effect blocked by A_1 receptor antagonists [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 [57].

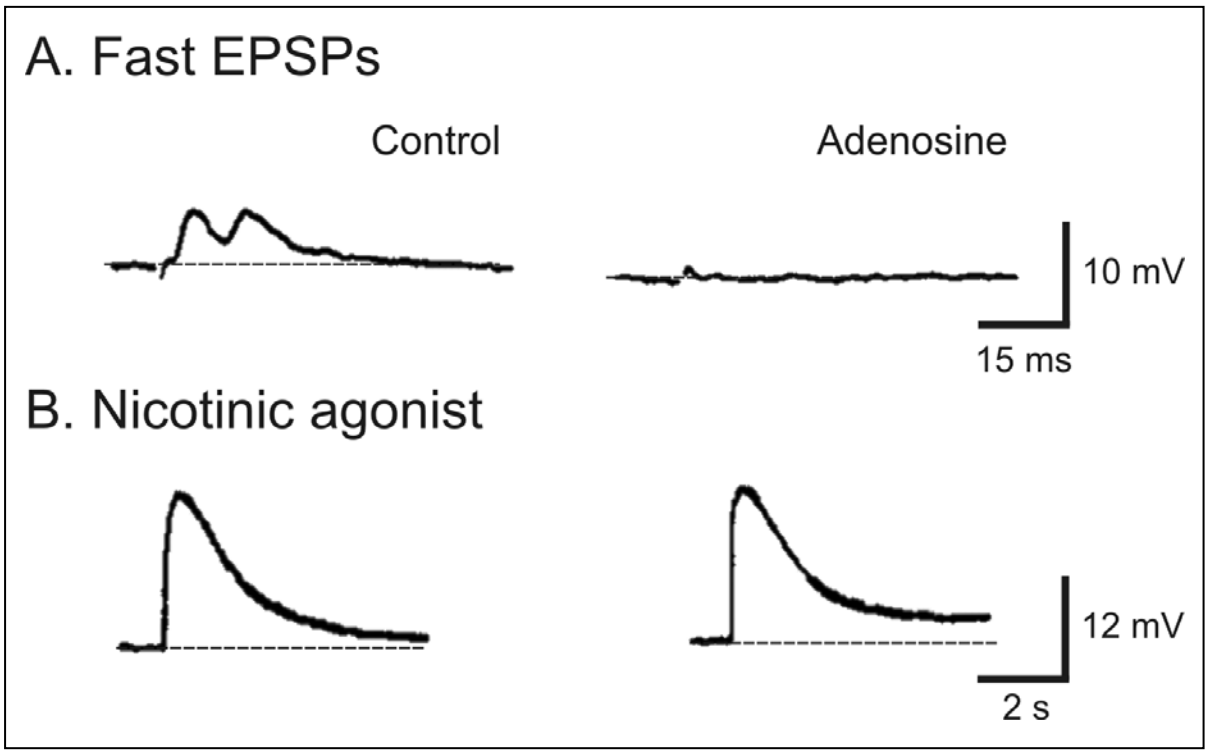

Figure 2. Presynaptic inhibition of fast synaptic transmission in an S neuron in the submucous plexus of guinea pig ileum. **A.** Left, a fast EPSP was evoked by a single stimulus to an intraganglionic fiber tract. Right, exposure to adenosine for 5 minutes suppressed the fast EPSP. **B.** Right, depolarization to pressure application of DMPP (1 μM). Left, during blockade of the fast EPSP, the depolarization to DMPP was unchanged in the presence of adenosine. Adapted from [58].

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 [22]. Unlike classical transmitters or neuropeptides, purines are located throughout a cell making standard histochemical approaches non-specific. 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.

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 [62]. In the myenteric plexus, ATP can be released from varicosities by nicotinic receptor agonists [63, 64]. Studies on the enteric circuitry point toward ATP serving primarily as a co-transmitter with VIP and NO [65]. For more information on the role of ATP in these reflexes [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 has, 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 non-selective permeability to cations. Inward currents induced by ATP acting at myenteric P2X receptors desensitize by 80% in 7 seconds [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 have 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].

In the rat, Xiang *et al* [77] 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 [77].

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, 78]. 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 [79, 80]. Evidence from combined electrophysiological and lesion studies have shown that some of these P2X receptors are in descending pathways [81]. These data have been extended by recent studies in the

submucosal plexus of the guinea pig where P2X mediated fast EPSPs have been identified [21]. Together these data suggest that endogenous ATP acting at P2X receptors mediates fast EPSPs between many neurons in the ENS.

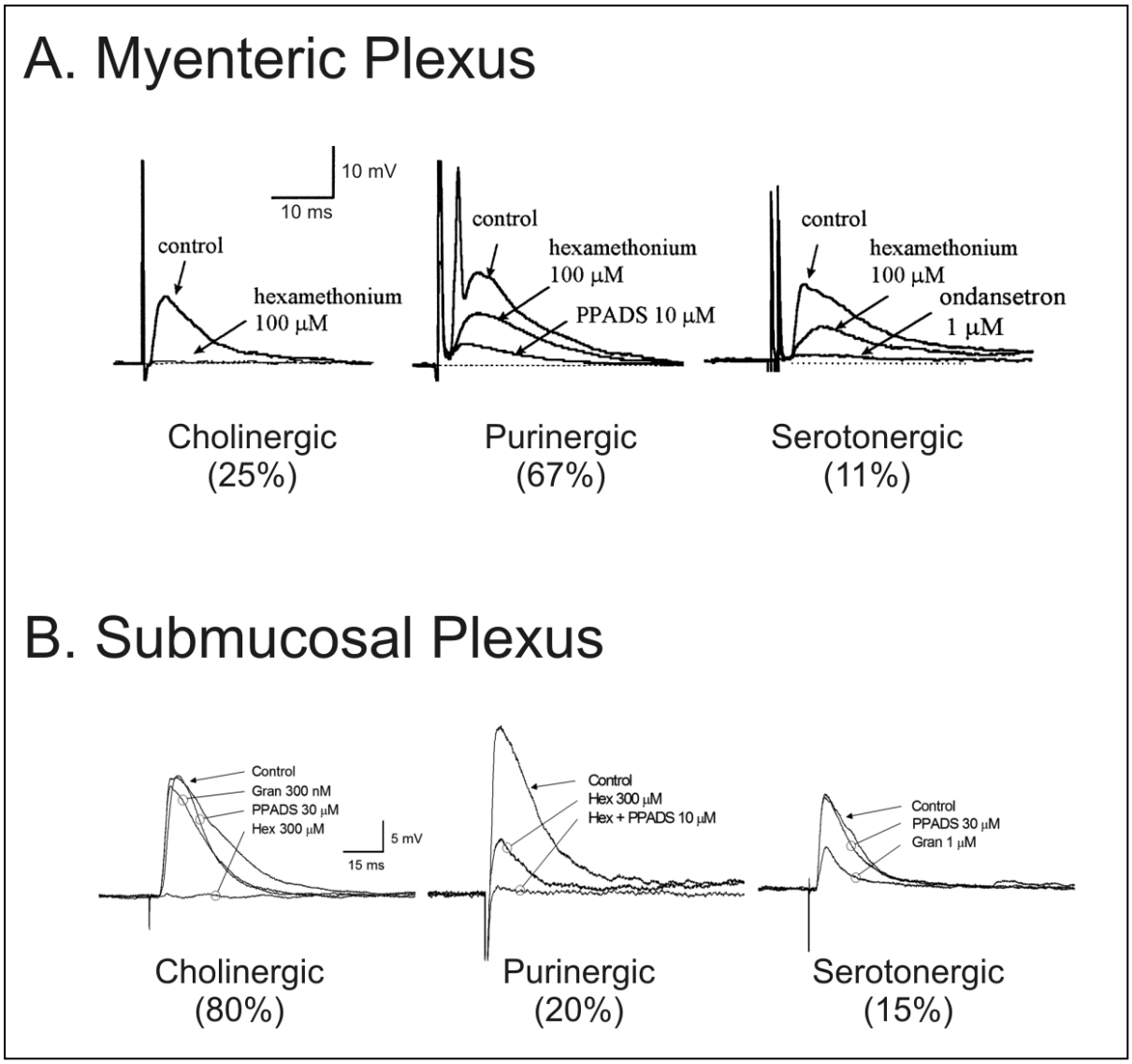

Figure 3. Fast EPSPs in the myenteric and submucous plexes of the guinea pig ileum have a prominent purinergic component. **A.** Pharmacologically distinct fast EPSPs from myenteric neurons. Left, a fast EPSP that was blocked by the nicotinic receptor antagonist, hexamethonium (100 μM). Middle, a fast 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 [82]. Copyright © Autonomic Neuroscience. **B.** Pharmacologically distinct fast EPSPs from submucosal neurons. 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 [21]. Copyright © Journal of Physiology. PERMISSION PENDING.

Intrinsic sensory neurons (AH neurons)

P2X receptors may also mediate activation of AH neurons and contribute to initiation of peristalsis [83]. ATP applied to AH cell bodies evoked a large depolarization in most AH neurons which was blocked by PPADS and, unexpectedly, potentiated by suramin. Similarly, application of ATP to the mucosal terminals of the AH neurons triggered action potentials that propagated back to the cell body.

Although $P2X_2$ receptors have been localized to AH neurons [84, 85], fast excitatory synaptic inputs are rarely recorded from these neurons in guinea pig [84] or mouse [85, 86]. Fast EPSPs that have been recorded from sensory neurons are much smaller in amplitude than in S neurons [11, 87]. Fast EPSPs in sensory neurons appear to be mediated by ACh acting at nicotinic receptors, but whether purines also participate in is not yet clear.

Sub-types of P2X receptor

One question remaining is what sub-types of P2X receptor are responsible for fast synaptic transmission in S neurons and the 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 [85, 88]. 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 [28, 89]. ATP failed to elicit a depolarization in S neurons from $P2X_2^{-/-}$ mice and fast EPSPs were not reduced by PPADS a non-specific P2 receptor antagonist (FIGURE 4A). Fast EPSPs from $P2X_3^{-/-}$ mice were reduced by PPADS to a similar level as were fast EPSPs from $P2X_3^{+/+}$ mice (FIGURE 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.

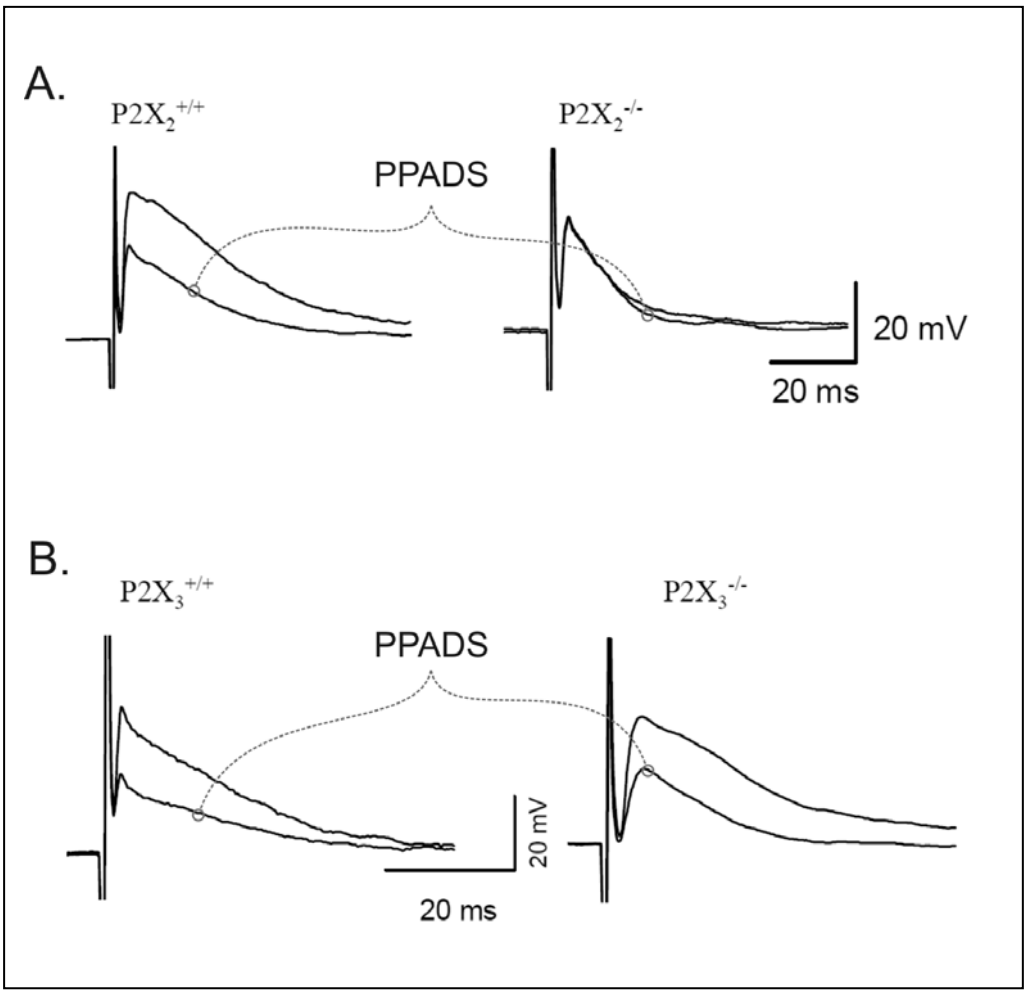

Figure 4. Fast EPSPs in myenteric S neurons from mice deficient in $P2X_2$ (A.) or $P2X_3$ (B.) receptors. **A.** Recordings from S neurons in $P2X_2^{+/+}$ (left) and $P2X_2^{-/-}$ (right) mice. The fast EPSPs recorded from neurons in tissues from $P2X2^{+/+}$ mice were inhibited by PPADS (10 μM - left) while those from neurons in $P2X2^{-/-}$ 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 [85, 88]. PERMISSION PENDING.

In a parallel study in the guinea pig ileum, all S neurons that were sensitive to ATP were depolarized by α,β-mATP but only 17% of AH neurons were sensitive to α,β-mATP [67]. Previous pharmacological studies have found that α,β-mATP sensitive P2X receptor subtypes ($P2X_1$ or $P2X_3$) contribute to fast EPSPs [79]. A selective antagonist for $P2X_1$ or $P2X_3$ receptors, TNP-ATP reduced ATP-induced depolarization as well as reducing fast EPSP amplitude in S neurons. These results are not, however, consistent with guinea pig myenteric neurons maintained in primary culture and murine myenteric neurons. Most of the neurons in primary culture do not desensitize to α,β-mATP and thus, there may be an alteration of $P2X_3$ receptors in culture conditions.

Together, these data suggest that in guinea pig, $P2X_3$ containing receptors in myenteric S neurons are responsible for mediating fast EPSPs while AH neurons 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.

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 [90, 91]. ATP is also a transmitter in descending excitatory reflexes [92, 93] as this pathway is sensitive to P2X receptor antagonists [see Chapter 2, this issue, 4]. Despite this, the

phenotype of either the $P2X_2$ or $P2X_3$ knockout mice does not show any outstanding abnormality [85, 88]. The mechanisms that maintain normal GI physiology when purinergic receptors are lost remains unclear.

P2Y receptors mediate sEPSPs in the ENS

Slow EPSPs in the ENS are long lasting (>1), moderate amplitude (~5 mV) depolarizations that generally require a train of presynaptic stimuli to evoke. 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 tackykinins 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]. More recently, the $P2Y_1$ receptor has been cloned from the guinea pig submucous plexus and characterized in human embryonic kidney cells [94], though the neuronal class expressing the receptor was not identified. Immunoreactivity for $P2Y_6$ was found in all regions of the

guinea pig gastrointestinal tract in the myenteric plexus, but not the submucous plexus [95]. About 32% of $P2Y_6$ receptor immunoreactivity was colocalized to NOS immunoreactive 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) [95] while $P2Y_2$ immunoreactivity colocalized with all the NPY immunoreactive neurons (secretomotor neurons) and a majority of calretinin immunoreactive neurons in the myenteric plexus (ascending interneurons and excitatory motor neurons) [96].

Properties of P2Y receptors in the ENS

Christofi *et al* [36], looking at cultured myenteric neurons from guinea pig, 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 and slow P2Y receptor mediated calcium rise and depolarization were identified [97]. In the intrinsic sensory neurons, this calcium release 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 [62].

Slow synaptic transmission

In guinea pig ileum, 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 [98]. Recent work in the myenteric plexus has found that S neurons have distension-evoked slow EPSPs that are blocked by PPADS [99]. These slow EPSPs only occurred in NOS-positive descending interneurons [100]. These data are supported by work in 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 (FIGURE 5) that are blocked by the selective $P2Y_1$ antagonist MRS 2179 [21, 101, 102]. Interestingly, a single stimulus evoked EPSP with a time course between that of a fast and a slow EPSP (an intermediate EPSP) was as shown to be blocked by PPADS, suramin and the selective $P2Y_1$ receptor antagonist MRS 2179 [21]. Most submucous neurons, including most non-cholinergic secretomotor neurons (VIP-IR) exhibited an intermediate EPSP which could occur spontaneously and was sometimes large enough to initiate action potentials.

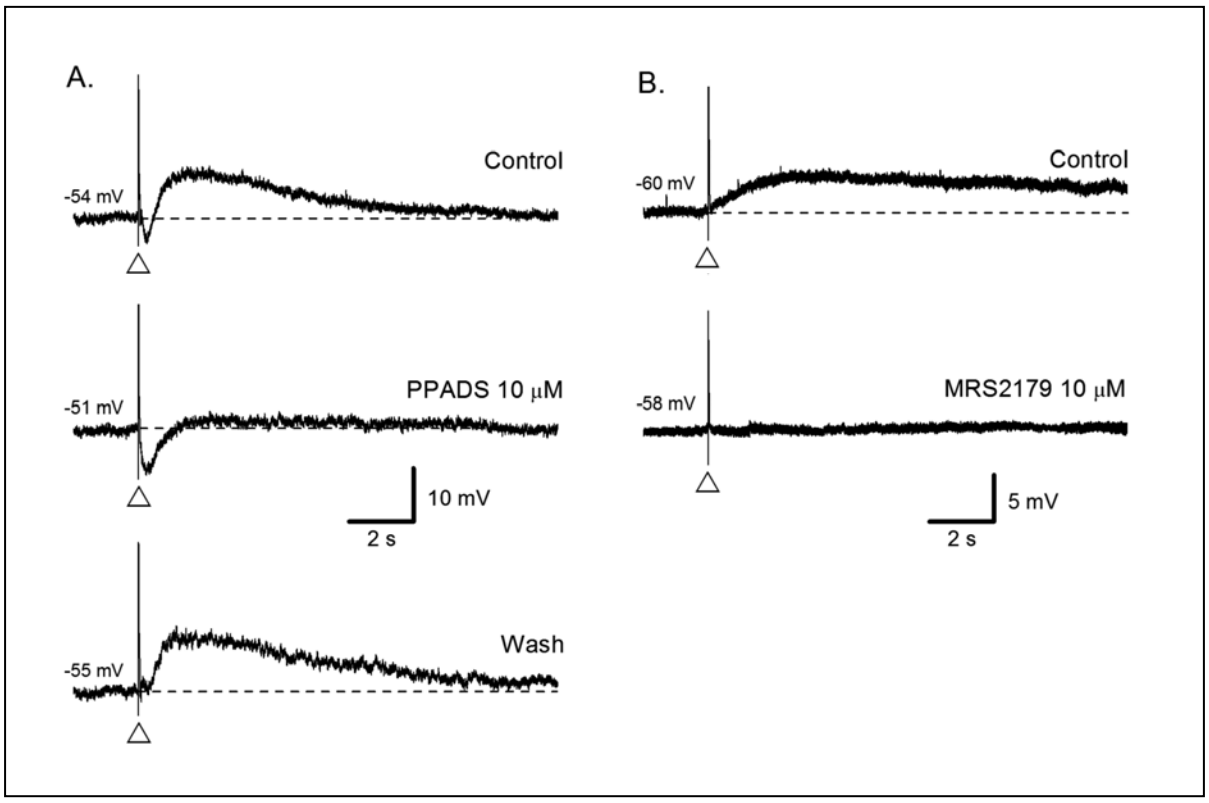

Figure 5. Slow EPSPs in the submucous plexus are blocked by P2Y receptor antagonists. Voltage traces taken from two submucosal neurons (A and B). **A.** A single pulse electrical stimulus evoked, in the following order: a fast EPSP, an intermediate EPSP, a small IPSP and a slow EPSP. Application of the 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) and the slow EPSP was abolished while the fast EPSP was spared. The IPSP in this cell had already been blocked with idazoxan. Adapted from [21].

Electrophysiological studied have also shown P2Y receptors on the intrinsic sensory neurons. Here they appear to mediate a hyperpolarization of the membrane due to an opening of the calcium activated potassium conductance [83, 98]. This has been supported by calcium imaging studies which suggest a P2Y receptor is coupled to release of internal calcium [36] possibly through a $P2Y_1$ receptor [62].

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 [103]. 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 [104]. One question that remains is what mechanism underlies the increase in purinergic fast EPSPs in inflamed tissue? Both upregulation of presynaptic ATP release or/and alteration of postsynaptic P2X receptors appear to contribute in the submucous and myenteric plexes, respectively [103, 104]. Recently this work has been extended to show that following resolution of colitis, the increase in purinergic fast EPSP amplitude remains [105] and that

there is an increase in fast EPSP amplitude in non-involved tissue remote from the inflammation [106].

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 has been found [107]. Other studies have shown that stimulation of A_{2A} receptors can reduce inflammation in the intestinal mucosa in rabbits and mice [108] and reduce tissue injury and inflammation in mice with toxin A-induced enteritis [109]. A recent study has utilized a high-density oligonucleotide microarray analysis to study TNBS-induced colitis [110]. They 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 [110].

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 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. Drugs targeted at these receptors have shown promise for ameliorating some of the changes seen during inflammation.

Acknowledgements

The authors wish to thank Dr Rebecca L. Bertrand for helpful comments and thank the Department of Physiology and Cell Biology, University of Nevada School of Medicine for financial support (PPB).

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