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REVIEW ARTICLE

The journey to establish purinergic signalling in the gut

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Abstract *Although the concept of purinergic signalling arose from experiments designed to find the identity of the non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmitter in the gut, it has taken many years for the more general importance of the various roles of ATP as a physiological messenger in the gut to be recognized. Firstly, vasoactive intestitial polypeptide (VIP) and later nitric oxide (NO) were considered the NANC transmitter and it was only later, after the concept of cotransmission was established, that ATP, NO and VIP were recognized as cotransmitters in NANC nerves, although the proportions vary in different gut regions. Recently, many purinoceptor subtypes have been identified on myenteric, submucosal motor, sensory and interneurons involved in synaptic neurotransmission and neuromodulation and reflex activity of several kinds, including ascending excitatory and descending inhibitory reflex pathways. Nucleotide receptors have been shown to be expressed on enteric glial cells and interstitial cells of Cajal. Purinergic mechanosensory transduction, involving release of ATP from mucosal epithelial cells during distension to stimulate subepithelial nerve endings of intrinsic and extrinsic sensory nerves to modulate peristalsis and initiate nociception respectively, is attracting current attention. Exciting new areas of interest about purinergic signalling in the gut include: involvement of purines in development, ageing and regeneration, including the role of stem cells; studies of the involvement of nucleotides in the activity of the gut of invertebrates and lower vertebrates; and the pathophysiology of enteric purinergic signalling in diseases including irritable bowel syndrome, postoperative ileus, oesophageal reflux, constipation, diarrhoea, diabetes, Chaga's and Hirschprung's disease.*

Keywords *Adenosine, ATP, cotransmission, gut development, irritable bowel syndrome, nociception, purinoceptor.*

Abbreviations: ACh, acetylcholine; CNS, central nervous system; DRG, dorsal root ganglion; 5-HT, 5-hydroxytryptamine; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; ICC, interstitial cells of Cajal; IJPs, inhibitory junction potentials; LSN, lumbar splanchnic; α,β-meATP, α,β-methylene ATP; NA, noradrenaline; NANC, non-adrenergic, non-cholinergic; NO, nitric oxide; NTS, nucleus tractus solitarius; PN, sacral pelvic; PPADS, pyridoxalphosphate-6-azonphenyl-2′,4′-disulphonic; UTP, uridine 5′-triphosphate; VIP, vasoactive intestinal polypeptide.

INTRODUCTION

There was early recognition by Langley of atropine-resistant responses of the gastrointestinal tract to parasympathetic nerve stimulation. However, it was not until the early 1960s that autonomic transmission other than adrenergic and cholinergic was established. In 1963, electrical activity was recorded in the guinea-pig taenia coli using the sucrose-gap technique and after stimulation of the intramural nerves in the presence of adrenergic and cholinergic blocking agents an inhibitory hyperpolarizing potential was observed by Burnstock *et al.*[1] Hyperpolarizing responses were blocked by tetrodotoxin, a neurotoxin that prevents the action potential in nerves without affecting the excitability of smooth muscle cells, indicating their neurogenic nature and establishing them as inhibitory junction potentials (IJPs) in response to non-adrenergic, non-cholinergic (NANC) nerve stimulation. In the late

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1960s, systematic studies were undertaken in an attempt to identify the transmitter utilized by the NANC nerves of the gut. Many substances were examined as putative transmitters in the NANC nerves of the gastrointestinal tract and bladder, but the substance that best satisfied the above criteria was the purine nucleotide, ATP.[2] Nerves utilizing ATP as their principal transmitter were subsequently named 'purinergic' and a tentative model of storage, release, and inactivation of ATP for purinergic nerves was proposed by Burnstock.[3] Since then much evidence has followed in support of the purinergic hypothesis,[4–6] although there was considerable opposition to this idea in the first decade or two after it was put forward. I believe that this was partly because biochemists felt that ATP was established as an intracellular energy source involved in various metabolic cycles and that such a ubiquitous molecule was unlikely to be involved in extracellular signalling. However, ATP was one of the first biological molecules to appear and, therefore, it is not surprising that it should have been used for extracellular, in addition to intracellular, purposes early in evolution.[7] The fact that potent ectoATPases were described in most tissues in the early literature was also a strong indication for the extracellular actions of ATP.[8]

Implicit in the concept of purinergic neurotransmission is the existence of postjunctional purinergic receptors, and the potent actions of extracellular ATP on many different cell types also implicate membrane receptors. Purinergic receptors were first defined in 1976 and 2 years later a basis for distinguishing two types of purinoceptor, identified as P1 and P2 (for adenosine and ATP/ADP respectively), was proposed by Burnstock.[9] At about the same time, two subtypes of the P1 (adenosine) receptor were recognized,[10] but it was not until 1985 that a proposal suggesting a pharmacological basis for distinguishing two types of P2 receptor (P2X and P2Y) was made.[11] In 1994, Abbracchio and Burnstock, on the basis of studies of transduction mechanisms and the cloning of nucleotide receptors, proposed that purinoceptors should belong to two major families: a P2X family of ligand-gated ion channel receptors and a P2Y family of G-protein-coupled receptors. This nomenclature has been widely adopted and currently seven P2X subtypes and eight P2Y receptor subtypes are recognized, including receptors that are sensitive to pyrimidines as well as purines.[12,13] Four subtypes of P1 G-protein-coupled receptors have been cloned, namely, A_1, A_{2A}, A_{2B}, and A_3. Seven subtypes of P2X receptors have been identified. The stoichiometry of $P2X_{1\text{-}7}$ receptor subunits is thought to involve three subunits that form a stretched trimer. Upon prolonged exposure of $P2X_7$ receptors to high concentrations of agonist, small cation channels and large channels or pores are activated that allow the passage of larger molecular weight molecules, leading to apoptosis. The P2X receptor family shows many pharmacological and operational differences.[14] Eight metabotropic P2Y receptor subtypes have been characterized ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$).[12] Many cells express multiple P2X and P2Y subtypes.[15] The pharmacology of purinergic signalling is complicated because both P2X and P2Y receptor subunits can combine to form either homomultimers or heteromultimers.[16]

COTRANSMISSION

Eccles introduced the term 'Dale's Principle' and the notion that neurons utilize a single transmitter then dominated thinking until the late 1970s. However, there were a number of hints in the literature that this might not be universally true and this led to a commentary by Burnstock[17] introducing the cotransmitter hypothesis. This hypothesis is now widely accepted and few neuroscientists today would venture to suggest that any neuron only utilizes one transmitter, although a principal transmitter might dominate for much of its life-span. There is now a substantial body of evidence to show that ATP is a cotransmitter with classical transmitters in most nerves in the peripheral and central nervous systems (CNS), although the proportions vary between tissues and species as well as in different developmental and pathophysiological circumstances.[5] There was early evidence for cotransmission in sympathetic nerves supplying the taenia coli.[18] Stimulation of periarterial sympathetic nerves led to release of tritium from guinea-pig taenia coli preincubated in [^{3}H]adenosine (which is taken up and converted largely to [^{3}H]ATP) and that the release of both tritium and noradrenaline (NA) was blocked by guanethidine. It has been claimed that ATP is the sole transmitter in sympathetic nerves supplying arterioles in the submucosal plexus of the intestine, while NA release from these nerves acts as a modulator of ATP release.[19] 'Axon reflex' activity is widespread in autonomic effector systems and forms an important physiological component of autonomic control of blood vessels and visceral organs, including the gut.[20] The early work of Holton[21] showing ATP release during antidromic stimulation of sensory collaterals, taken together with the evidence for glutamate in primary afferent sensory neurons, suggests that ATP and glutamate may be cotransmitters in these nerves. Most enteric neurons are derived from

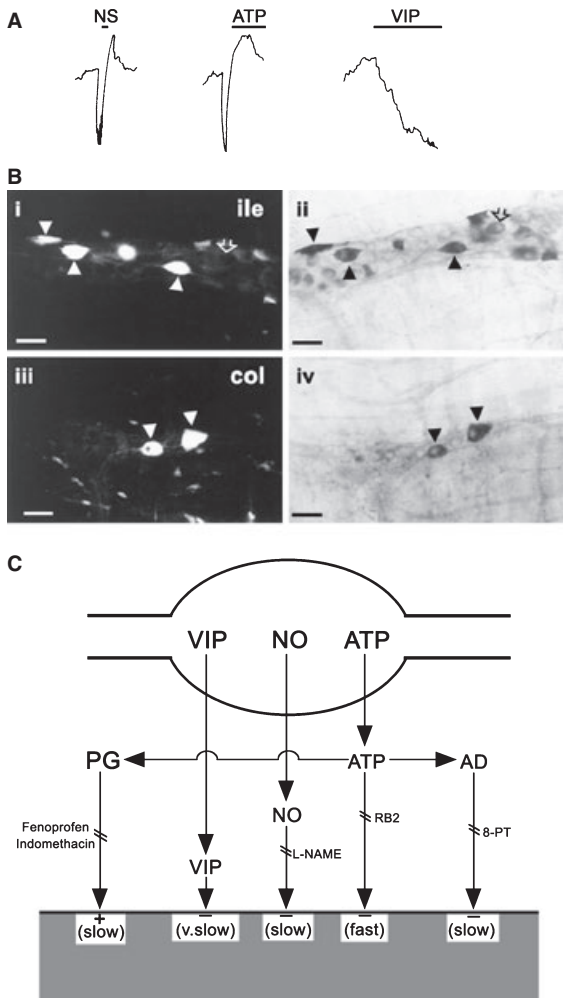

Figure 1 A: The responses of the isolated taenia coli to intramural nerve stimulation (NS, pulse width of 0.3 ms, supramaximal voltage and frequency of 0.4 Hz for 10 s), ATP (0.7 μmol L^{-1}) and VIP (0.6 μmol L^{-1}). Guanethidine (3.4 μmol L^{-1}) was present throughout. [From Ref. (77) with permission from Elsevier]. B: Micrographs showing colocalization of ATP and NADPH-diaphorase in myenteric ganglion neurons of ileum and proximal colon of the rat: i, quinacrine-fluorescent myenteric neurons of ileum (ile): ii, NADPH-diaphorase-positive myenteric neurons of the same preparation. Most of the fluorescent neurons in (i) also contain NADPH-diaphorase (arrowheads), but there are some NADPH-diaphorase-positive but quinacrine-negative neurons (open arrows). iii, Quinacrine-fluorescent neurons in the myenteric plexus of rat proximal colon (col); iv, NADPH-diaphorase-positive myenteric neurons of the same preparation (iii). Note that all quinacrine-fluorescent neurons also contain NADPH-diaphorase (arrowheads). Calibration bars = 30 μm. [From Ref. (78) with permission from Springer]. C: Schematic representation of non-adrenergic non-cholinergic (NANC) inhibitory nerves in the gut. Neurotransmitters and/or agonists: VIP, vasoactive intestinal polypeptide; NO, nitric oxide; ATP, adenosine 5′-triphosphate; AD, adenosine; PG, prostaglandins. Antagonists or inhibitors: L-NAME, N^G-nitro-L-aginine methyl ester; RB2, reactive blue 2; 8-PT, 8-phenyltheophylline. Responses: +excitatory; −inhibitory. [From Ref. (23) with permission from Springer].

neural crest tissue that differs from that which forms the sympathetic and parasympathetic systems and appear to represent an independent local control system. Cotransmission occurs in enteric neurons and the concept of 'chemical coding' was proposed.[22] A subpopulation of intramural enteric nerves provides NANC inhibitory innervation of gastrointestinal smooth muscle. Three major cotransmitters are released from these nerves: (i) ATP producing fast IJPs; (ii) nitric oxide (NO) also producing IJPs, but with a slower time course; and (iii) vasoactive intestinal polypeptide (VIP) producing slow tonic relaxations[23] (see Fig. 1). The proportions of these three transmitters vary considerably in different regions of the gut and in different species. For example, in some sphincters, the NANC inhibitory nerves primarily utilize VIP, in others they utilize NO, and in non-sphincteric regions of the intestine, ATP is more prominent. Recently ATP and NO have been shown to co-mediate NANC relaxation of the circular muscle of the human sigmoid colon.[24]

ENTERIC GANGLIONIC NEURONS: SYNAPTIC TRANSMISSION AND NEUROMODULATION

The first studies of the effects of ATP on single myenteric neurons from guinea-pig small intestine using the intracellular electrophysiological recording technique were in the late 1980s.[25] Myenteric neurons are classified into two groups electrophysiologically and ATP elicits hyperpolarization in 80% of AH (type II) neurons and depolarization in 90% of S (type I) neurons in a dose-dependent manner. Subsequently, several laboratories extended these studies of purinergic signalling in guinea-pig myenteric and submucous neurons including elegant whole-cell and outside-out patch-clamp recording.[26–35]

Nicotinic acetylcholine (ACh) and P2X receptors play a central role in fast synaptic excitatory transmission in the myenteric plexus (Fig. 2). Nicotinic receptors on S type neurons on the guinea-pig intestine are composed of at least the α3 and β4 subunits, whereas P2X receptors in S type neurons are composed of $P2X_2$ subunits. ATP acting on $P2X_2$ receptors is the predominant fast excitatory neurotransmitter in the descending pathways. ATP regulates synaptic transmission by pre- as well as postsynaptic modulation mechanisms in guinea-pig myenteric neurons. Prejunctional modulation of ACh release from peripheral cholinergic nerves

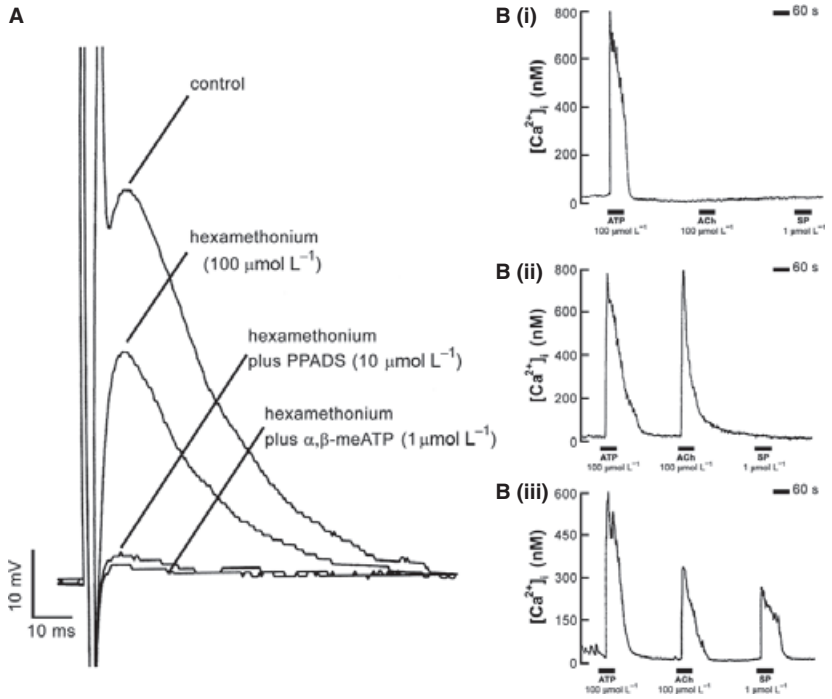

Figure 2 A: Effect of purinergic P2X agonist α,β-methylene ATP (α,β-meATP, 1 μmol L^{-1}) on the non-cholinergic component of the fast excitatory postsynaptic potential (fEPSP) of guinea-pig myenteric neurons. The non-cholinergic component was totally blocked by pyridoxalphosphate-6-azonphenyl-2′,4′-disulphonic (PPADS) (10 μmol L^{-1}). The neuron was allowed to recover in the presence of hexamethonium (not shown). Superfusion with α,β-meATP also totally blocked the non-cholinergic component of the fEPSP. (Reproduced from Ref. 26 with permission of Elsevier). B(i)–(iii): Representative traces from three cultured myenteric neurons of guinea-pig illustrate the heterogeneity of responses to agonist combinations. The *x*-axis is time in seconds and lines on this axis represent exposure to agonists. The *y*-axis is $[Ca^{2+}]_i$ in nmol L^{-1}. Buffer is superfused during the periods between agonist exposure. Neurons were from the same coverslip and the same experiment but differ in responses of $[Ca^{2+}]_i$ to superfusion with ATP, acetylcholine (ACh) and substance P (SP). Each agonist was superfused for 60 s followed by buffer perfusion for 300 s. [Reproduced from Ref. (79) with permission of Elsevier].

by adenosine was observed in the isolated guinea-pig ileum and the myenteric plexus longitudinal muscle preparation. More recently evidence has been presented for a prejunctional modulatory action by ATP itself, as well as adenosine. Purine nucleotides and nucleosides can also act on postjunctional receptors to modulate cholinergic and adrenergic neurotransmission. ATP augments nicotinic fast depolarization produced by ACh, but inhibits muscarinic and SP-mediated depolarizations in both AH and S neurons. Exogenous and endogenous ATP, released during increase in intraluminal pressure, inhibits intestinal peristalsis in guinea-pig via different apamin-sensitive purine receptor mechanisms. Exogenous ATP depresses peristalsis mostly via suramin- and pyridoxalphosphate-6-azonphenyl-2′,4′-disulphonic (PPADS)-insensitive $P2X_4$ receptors, whereas endogenous purines act via $P2X_2$ and/or $P2X_3$ and/or $P2X_{2/3}$ receptors sensitive to both suramin and PPADS to initiate peristalsis. ATP plays a major role 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. Descending inhibitory reflexes involve P2X receptor-mediated transmission from interneurons to motor neurons in guinea-pig ileum. Experiments with $P2X_2$ and $P2X_3$ receptor knockout mice showed that peristalsis is impaired in the small intestine. Fast excitatory postsynaptic potentials occur in bursts in the myenteric plexus during evoked motor reflexes in the guinea-pig ileum. Synaptosomal preparations from the guinea-pig ileum myenteric plexus were first described in the early 1980s and ATP and adenosine were equipotent in their ability to inhibit the nicotinically induced release of [^{3}H]ACh. Intracellular recordings from submucosal neurons in guinea-pig small intestine showed that ATP induced fast transient depolarization of most AH-type neurons and fast transient depolarization followed by slower onset, longer lasting depolarization of S-type neurons. The

functional interaction between nicotinic and P2X receptors has been investigated in freshly dissociated guinea-pig submucosal neurons in primary culture: whole-cell currents induced by ATP were blocked by PPADS and showed some interdependence on ACh-induced nicotinic currents blocked by hexamethonium (Fig. 2A). Slow excitatory postsynaptic potentials were mediated by $P2Y_1$ receptors in neurons in the submucosal plexus of guinea-pig small intestine. Immunohistochemical studies have demonstrated $P2X_2$, $P2X_3$ and $P2X_7$ receptors in subpopulations of guinea-pig and rat myenteric and submucous ganglion neurons. $P2X_2$ receptors are also expressed by neurons in the mouse myenteric and submucous plexuses and $P2X_5$ receptors on nerve fibres that envelop ganglion cell bodies and possibly glial cell processes. Cross-inhibitory interactions between γ-amino butyric acid A and P2X receptor channels in myenteric neurons of guinea-pig small intestine have been described.

There is growing evidence for the expression of P2Y receptors on enteric neurons in addition to P2X receptors.[36–40] Fast and slow depolarizations and Ca^{2+} responses of cultured guinea-pig ileal submucosal neurons to ATP were mediated by P2X and $P2Y_1$ receptors respectively. In the mouse gastrointestinal tract, $P2Y_1$ receptors on NANC myenteric neurons appear to mediate relaxation through NO and ATP. A $P2Y_1$ receptor has been cloned and characterized from guinea-pig submucosa. $P2Y_2$ receptors are widely distributed on S-type (Dogiel type 1) neurons in the myenteric and submucosal plexuses throughout the guinea-pig gut. About 40–60% of $P2X_3$ receptor-immunoreactive neurons were immunoreactive for $P2Y_2$ receptors in the myenteric plexus and all $P2X_3$ receptor-immunoreactive neurons expressed $P2Y_2$ receptors in the submucosal plexus. It has been shown that 30–36% of the ganglion cells in the myenteric, but not submucosal plexus of the guinea-pig gastrointestinal tract, are labelled with $P2Y_6$ receptor-immunoreactive neurons. About 42–46% of the neurons in both myenteric and submucosal plexuses are immunoreactive for $P2Y_{12}$ receptors; about 28–35% of $P2Y_6$ receptor-immunoreactive neurons coexist with NO synthase, but not with calbindin, while all $P2Y_{12}$ receptor-immunoreactive neurons were immunopositive for calbindin and appear to be AH intrinsic primary afferent neurons. In a recent study of the rat distal colon, $P2Y_1$ and $P2Y_6$ immunoreactivity was found on smooth muscles, $P2Y_4$ and $P2Y_6$ receptor immunoreactivity on glial cells in both plexuses, $P2Y_4$ receptors on interstitial cells of Cajal (ICC), while $P2Y_2$ and $P2Y_{12}$ receptors were demonstrated on enteric neurons. Differential gene expression of A_1, A_{2A}, A_{2B} and A_3 receptors in human enteric neurons has been reported,[41] and fine-tuning modulation of myenteric motoneuron activity by endogenous adenosine has been claimed.[42]

In summary, the roles of P1, P2X and P2Y receptor subtypes in synaptic transmission and neuromodulation involved in reflux pathways in the myenteric and submucous plexuses have been explored only relatively recently, but they clearly play major roles in these activities.

INTRAMURAL ENTERIC SENSORY NEURONS

The after hyperpolarization (AH) defined neurons appear to be the enteric sensory neurons, which represent about 30% of the neurons in the myenteric plexus. About 90% of Dogiel type II neurons in the guinea-pig ileum exhibit slow after hyperpolarizations.[43] These neurons are distinct from Dogiel type I, S neurons, which are motor neurons or interneurons. $P2X_3$ receptors are dominant on neurons in the submucosal plexus of the rat ileum and distal colon and up to about 60% of the neurons express calbindin, a marker for enteric sensory AH neurons.[33] $P2X_3$ receptor-immunoreactivity has also been shown on sensory neurons in the human myenteric plexus.

ATP and α,β-methylene ATP (α,β-meATP) activated submucosal terminals of intrinsic sensory neurons in the guinea-pig intestine supporting the hypothesis of Burnstock[44] that ATP released from mucosal epithelial cells has a dual action on $P2X_3$ and/or $P2X_{2/3}$ receptors in the subepithelial sensory nerve plexus and that these receptors may contribute to the detection of distension or intraluminal pressure increases and initiation of reflex contractions.[45] Single fibre analysis showed that ATP acts on the terminals of low threshold intrinsic enteric sensory neurons to initiate or modulate intestinal reflexes and acts on the terminals of high threshold extrinsic sensory fibres to initiate pain. Further support comes from the demonstration that peristalsis is impaired in the small intestine of mice lacking the $P2X_3$ subunit[29] and that up to 75% of the neurons with $P2X_3$ receptor immunoreactivity in the rat submucosal plexus expressed calbindin.[33]

Purinergic mechanosensory transduction has also been implicated in reflex control of intestinal secretion, whereby ATP released from mucosal epithelial cells acts on $P2Y_1$ receptors on enterochromaffin cells to release 5-hydroxytryptamine (5-HT), which leads to regulation of secretion either directly or via intrinsic reflex activity.[46]

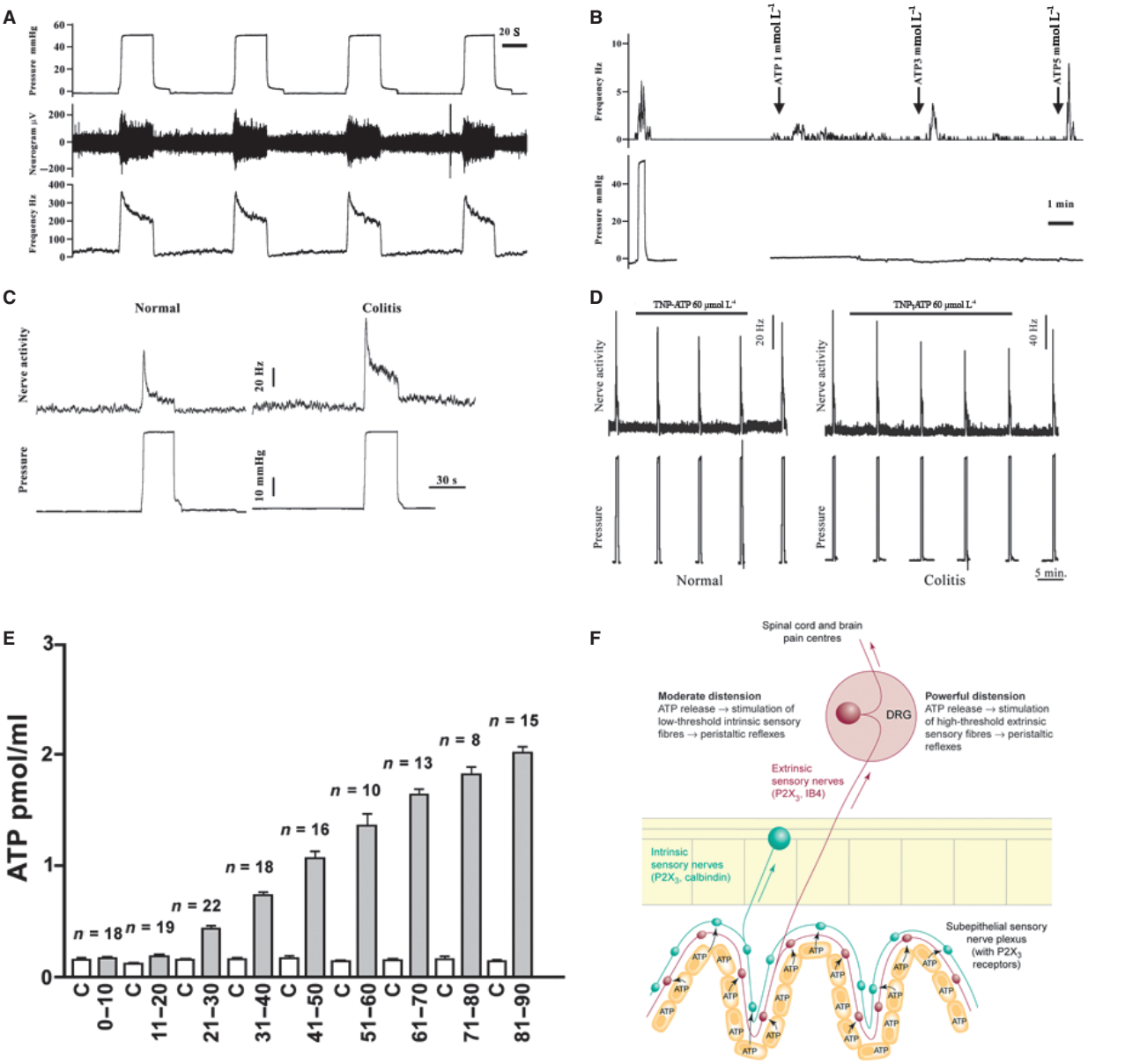

Figure 3 A: Repeated phasic distentions to 50 mmHg in the rat colorectum. (Top) Intraluminal pressure (mmHg); (middle) pelvic afferent nerve activity (μV); (bottom) frequency of spikes (Hz). B: Single-unit analysis shows that fibres responding to distention are also activated by ATP in a dose-dependent manner. (Top) Frequency of single-unit firing (Hz); (bottom) pressure (mmHg). C: Sample recordings from the pelvic nerve in a normal colorectal preparation and a colitis model. Single-unit analysis confirmed that both preparations have the same number of active nerve fibres. Background activity and response to 50-mmHg distension are increased in the colitis model, demonstrating a greater firing rate per unit. D: Pelvic nerve responses to distension (50 mmHg) in the presence of the P2X receptor antagonist 2′,3′-*O*-trinitrophenyl-ATP (TNP-ATP, 60 μmol L^{-1}). Colitis models show smaller responses to distension in the presence of the antagonists. E: ATP concentration of luminal fluid samples from the rat colorectum during distention. Each column shows the mean ATP release (pmol mL^{-1}) ± SEM for each of the pressure groups listed (mmHg). Control samples were collected before each distention (C). The numbers above the columns refer to the number of distentions in each pressure group. F: A schematic illustrating the hypothesis about purinergic mechanosensory transduction in the gut. It is proposed that ATP released from mucosal epithelial cells during moderate distension acts preferentially on $P2X_3$ receptors on low-threshold subepithelial intrinsic sensory nerve fibres (labelled with calbindin), contributing to peristaltic reflexes. ATP released during extreme distension also acts on $P2X_3$ receptors on high threshold extrinsic sensory nerve fibres [labelled with isolectin B4 (IB4)] that send messages via the dorsal root ganglia (DRG) to pain centres in the CNS. [A, B and E reproduced from Ref. (45) with permission from Elsevier; C and D reproduced from Ref. (70) with permission from the American Physiological Society; and F reproduced from Ref. (80) with permission from Elsevier].

SECRETOMOTOR NEURONS

The presence of intrinsic neurons in the enteric plexus controlling secretion in mucosal epithelial cells has been recognized for a long time with both cholinergic and non-cholinergic secretomotor neurons involved. In general, extrinsic parasympathetic activity increases intestinal secretion, while inhibition occurs with sympathetic stimulation. ATP has been shown to modulate gastric mucous and acid secretion as well as intestinal secretion and both P2Y and P2X receptors have been identified on mucosal epithelial cells and gastric glands.[23] Extracellular ATP and adenosine have established roles as potent stimulants of fluid and electrolyte secretion in colon, gall bladder, pancreatic duct and bile duct, were it seems likely to be released from both local cells and nerves. ATP-induced contractions of muscularis mucosae evoked colonic epithelial secretion via prostaglandin synthesis and non-cholinergic secretormotor nerve stimulation. Intrinsic enteric sensory neurons also provide direct innervation of the mucosa and stroking the mucosal lining of the guinea-pig colon with a brush releases ATP that activates $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors to trigger an intestinal neural reflex and an increase in short-circuit current, indicative of chloride secretion.[47] ATP released as an enteric neurotransmitter acts on $P2Y_1$ excitatory receptors on intestinal secretomotor neurons in the guinea-pig to evoke neurogenic mucosal secretion.[48] Studies with $P2Y_2$ and $P2Y_4$ receptor knockout mice indicate that both these receptors are present in the luminal membranes of mouse distal colonic mucosa and that stimulation of these receptors leads to K^+ secretion.[49]

In summary, the intrinsic secretomotor neurons, particularly those on the submucous plexus, modulate chloride, potassium, mucous and acid secretion via P1 and P2Y receptors expressed by mucosal epithelial cells. ATP released from these cells also appears to initiate intestinal secretary refluxes via P2Y receptors on subepithelial sensory nerve terminals and $P2Y_1$ receptors on secretomotor neuron cell bodies may also be involved.

INTERSTITIAL CELLS OF CAJAL AND ENTERIC GLIAL CELLS

Interstitial cells of Cajal are a specialized cell type that control the activities of smooth muscle cells in the gut. They have been shown to be innervated by enteric nerves. $P2X_2$ and $P2X_5$ receptors are expressed on ICC in guinea-pig intestine[50] and more recently $P2Y_4$ receptors have also been identified on ICC cells in guinea-pig gastrointestinal tract to modulate intracellular Ca^{2+} oscillations.[51] These observations are consistent with ATP being released as a cotransmitter from enteric nerves to regulate the activities of these cells. Purinergic modulation of pacemaker $[Ca^{2+}]_i$ activity in ICC is mediated by P2X receptors.[52]

Enteric glia, which out-number enteric neurons 2 : 1, display morphological and molecular similarities to CNS astrocytes and stain for glial fibrillary acidic protein. They were first shown in 1996 to respond to ATP and uridine 5′-triphosphate (UTP) via P2 receptors by an increase in intracellular calcium, probably via $P2Y_2$ or $P2Y_4$ receptors and later supported by evidence for release of Ca^{2+} from intracellular stores. Immunohistochemical studies also showed expression of $P2X_7$ receptors on S100 immunolabelled enteric glial cells[53] and $P2Y_4$ receptors.[51] Ectonucleotide NTPDase2 has been shown to be exclusively localized to the surface of enteric glial cells, suggesting that enteric glia control the availability of ATP and UTP.[54] There is indirect evidence that enteric glia may release ATP, to participate in the intercellular propagation of Ca^{2+} waves between enteric glial cells and Ca^{2+} wave-induced ATP release was shown to elicit neuronal responses.

In summary, ICC have a major role as pacemakers for spontaneous smooth muscle activity and the presence of both P2X and P2Y receptors on these cells suggests that ATP, either released as a cotransmitter from the nerves that supply ICC, or released locally in response to stretch, may contribute to modulation of the activities of these cells. The roles of $P2Y_2$, $P2Y_4$ and $P2X_7$ receptors on enteric glial cells is interesting and, by analogy with what is known of neuron–glial cell interactions in the CNS (see the following section) may play comparable roles in the gut, but this remains to be examined.

EXTRINSIC ENTERIC NERVES AND CENTRAL NERVOUS SYSTEM CONTROL

Although the enteric nervous system can function independently of the CNS, the latter has a major role in co-ordinating the activity of the gut through both sympathetic and parasympathetic motor as well as sensory pathways. The nucleus tractus solitarius (NTS) (particularly neurons in the caudal NTS) is a central relay station for viscerosensory information to digestive neuronal networks, while efferent fibres supplying the gut originate in the dorsal motor nucleus of the vagus. The parasympathetic motor pathways consist of the vagus nerve that controls motor and secretomotor function in the upper gastrointestinal tract, while the sacral nerves regulate functions of the distal colon and rectum. Sympathetic fibres enter the gut at intervals with their origin in cell bodies in the prevertebral

ganglia; they supply blood vessels and sphincters and modulate enteric reflexes at the enteric plexus level. Primary afferent sensory neurons that carry information to the CNS are present in vagal and splanchnic nerves; vagal sensory fibres have cell bodies in the nodose ganglion, while splanchnic sensory nerves have their cell bodies in dorsal root ganglia.

Purinergic mechanisms are involved at most levels in these pathways. Nucleotide receptors are prominent in the brainstem areas and spinal cord regulating gastrointestinal function, particularly the NTS, nucleus ambiguus, dorsal vagal complex and area postrema.[5] Intraganglionic laminar nerve endings are specialized mechanosensory endings of vagal afferent nerves in the rat stomach. They arise from the nodose ganglion; they express both $P2X_2$ and $P2X_3$ receptors and are probably involved in physiological reflex activity, especially in early postnatal development.[55,56] A subpopulation of nodose vagal afferent nociceptive nerves sensitive to $P2X_3$ receptor agonists was later identified and shown to be different from the non-nociceptive vagal nerve mechanoreceptors. ATP has been shown to be a cotransmitter in both preganglionic and postganglionic parasympathetic nerve fibres and in sympathetic nerves supplying the gut. In particular, ATP has been identified as the principal transmitter in sympathetic nerves supplying small arteries supplying the intestine and submucosal arterioles.[19]

A hypothesis was proposed suggesting that purinergic mechanosensory transduction in the gut initiated both physiological reflex modulation of peristalsis via intrinsic sensory fibres and nociception via extrinsic sensory fibres.[44] Evidence in support of this hypothesis was obtained from a rat pelvic sensory nerve-colorectal preparation.[45] Distension of the colorectum led to pressure-dependent increase in release of ATP from mucosal epithelial cells and also evoked pelvic nerve excitation. This excitation was mimicked by application of ATP and α,β-meATP and attenuated by the selective $P2X_3$ and $P2X_{2/3}$ antagonist, trinitophenol-ATP and by PPADS. The sensory discharge was potentiated by ARL-67156, an ATPase inhibitor. Single fibre analysis showed that high threshold fibres were particularly affected by α,β-meATP. Lumbar splanchnic (LSN) and sacral pelvic (PN) nerves convey different mechanosensory information from the colon to the spinal cord. Forty per cent of LSN afferents responded to α,β-meATP compared to only 7% of PN afferents. Thirty-two per cent of retrogradely labelled cells in the mouse dorsal root ganglion (DRG) at levels T8-L1 and L6-S1, supplying sensory nerve fibres to the mouse distal colon, were immunoreactive for $P2X_3$ receptors. Extrinsic and possibly intrinsic sensory nerves associated with mucosal epithelial cells appear to be sensitive to pH, probably via $P2X_2$ and $P2X_{2/3}$ receptors. The $P2X_3$ receptor subtype predominates in AH type neurons and probably participates in mechanosensory transduction.[57]

Purinergic mechanosensory transduction has also been implicated in reflex control of secretion, whereby ATP released from mucosal epithelial cells acts on $P2Y_1$ receptors on enterochromaffin cells to release 5-HT, which leads to regulation of secretion either directly or via intrinsic reflex activity.[58]

PATHOPHYSIOLOGY OF PURINERGIC SIGNALLING IN THE GUT

The excitability of visceral afferent nerves is enhanced following injury, ischaemia and during inflammation, as for example in irritable bowel syndrome (IBS). Under these conditions, substances are released from various sources that often act synergistically to cause sensitization of afferent nerves to mechanical or chemical stimuli. Receptors to these substances (including ATP) represent potential targets for drug treatment aimed at attenuating the inappropriate visceral sensation and subsequent reflex activities that underlie abnormal bowel function and visceral pain.[59,60] α,β-Methylene ATP was shown to stimulate mechanosensitive mucosal and tension receptors in mouse stomach and oesophagus leading to activity in vagal afferent nerves. The sensitizing effects of $P2X_3$ receptor agonists on mechanosensory function are induced in oesophagitis.[61] Enhanced activity in purinergic pathways occurs in postoperative ileus, but is reversed by orphanin FQ. Recent reviews have highlighted the potential of purinergic drugs for the treatment of functional gastrointestinal tract disorders.[23,59,60]

Inflammatory bowel disease

Extracellular nucleotides and their receptors have been implicated in the pathogenesis of inflammatory bowel disease (IBD). T lymphocytes are thought to play a primary role in the induction of epithelial cell damage in IBD and the $P2Y_6$ receptor was found by this group to be highly expressed on the T cells infiltrating the diseased segment, but absent in T cells of unaffected bowel. This suggests that $P2Y_6$ receptor and its selective agonist, UDP, may play a role in the pathogenesis of IBD. Later papers have shown that $P2Y_6$ receptors are involved in monocytic release of interleukin-8 and stimulation of NaCl secretion. During inflammation of the gastrointestinal tract, glial cells proliferate and produce cytokines; thus, $P2X_7$ receptors may play a

role in the response of enteric glia to inflammation.[53] Functional expression of the $P2X_7$ receptor in colonic macrophages and T lymphocytes in the mucosa of IBD suggests they may play a role in the immunopathology of the disease.[62] During chronic interstitial inflammation induced by infection of mice with the parasite *Schistosoma mansoni* for 16 weeks, purinergic modulation of cholinergic nerve activity was impaired.[63] Intestinal epithelial cells from patients with cystic fibrosis fail to consistently conduct Cl^- in response to ATP and UTP that elevate intracellular Ca^{2+} and this may be of value in the design of treatments to ameliorate gastrointestinal symptoms of cystic fibrosis. A relationship between the enteric nervous system and inflammation-induced mucosal transport responses was demonstrated by experiments in which neural blockade abolished the secretory response induced by mast cell degranulation and neutrophil activation and new approaches targeting the enteric nervous system show promise for the treatment of secretory diarrhoea.[64] Enteric P2X receptors have been proposed as potential targets for the treatment of IBS.[65] Gastric ulcers evoke hyperexcitability and enhance P2X receptor function in rat gastric sensory neurons, thereby potentially contributing to the development of dyspeptic symptoms.[66]

Diabetes

Electrical recording from gastric smooth muscle from streptozotocin-induced diabetic rats during transmural nerve stimulation showed IJPs of reduced amplitude and no excitatory junction potentials. The hyperpolarizations in response to ATP were similar in the circular muscle of the caecum of streptozotocin diabetic (8 weeks) and untreated control rats, although the rate of hyperpolarization of single IJPs was slower in the diabetic tissues.[67] While ATP-induced relaxations of longitudinal strips from the gastric fundus were not significantly different in control and diabetic rats, the stimulation-induced release of ATP increased threefold. Desensitization of receptors to ATP with α,β-meATP reduced the relaxant responses to both ATP and electrical field stimulation, suggesting a role for ATP in NANC neurotransmission in rat gastric fundus and this reduction was greater in diabetic tissues.[68] In view of these data, it was suggested that the purinergic component of the vagal NANC responses of the stomach may be increased in diabetes, a finding reminiscent of an increased purinergic component in parasympathetic control of bladder in interstitial cystitis and in sympathetic nerves supplying blood vessels in spontaneously hypertensive rats. While maximum relaxant responses and sensitivity of the colon to ATP were unchanged in 8-week streptozotocin diabetic rats, the responses to adenosine were reduced.[69]

Nociception

Intrinsic sensory neurones in the submucous plexus of the gut, as well as extrinsic sensory nerves, show positive immunoreactivity for $P2X_3$ receptors.[45] It has been proposed by Burnstock[44] that during excessive (colic) distension, high-threshold extrinsic enteric sensory fibres are activated via $P2X_3$ receptors by ATP released from mucosal epithelial cells, leading to initiation of nociceptive impulses that pass messages through the DRG to pain centres in the CNS (Fig. 3).[56] $P2X_3$ purinergic signalling enhancement in an animal model of colonic inflammation has been described, due, at least in part, to the appearance of $P2X_3$ receptor expression in a greater number of calcitonin gene-related peptide-labelled small nociceptive neurons in the DRG.[70] $P2X_3$ receptor expression is increased in the enteric plexuses in human IBD suggesting a potential role in dysmotility and pain.[71]

Motility disorders

Bile induces ATP depletion and contributes to the early mucosal permeability alteration and barrier lesions that occur during experimental oesophageal reflux.[72] P2Y receptors on smooth muscle and ATP production in myenteric neurons increase in postoperative ileus, probably contributing to delayed colonic transit.[73] It has been suggested that agonists acting on P2X receptors on intrinsic enteric neurons may enhance gastrointestinal propulsion and secretion and that these drugs might be useful for treating constipation-predominant IBS, while P2X antagonists might be useful for treating diarrhoea-predominant IBS.

Hirschsprung's and Chaga's diseases

In aganglionic intestine in Hirschsprung's disease, there was only weak $P2X_3$ receptor immunostaining in the myenteric and submucous plexuses compared to normal intestine.[74] This finding is consistent with experimental studies that reported that no IJPs could be evoked in smooth muscle by intramural nerve stimulation of the rectosigmoidal part of the large intestine of Hirschsprung's patients, and ATP caused contraction of the muscle.[75]

In Chaga's disease, enhancement of $P2X_7$ receptor-associated cell permeabilization during the acute phase of the disease was reported,[76] although purinergic signalling through other P2X receptor subtypes and

P2Y receptors also seems to be impaired, perhaps because the parasite protozoan that causes the disease contains high levels of ATPases.

CONCLUSIONS AND FUTURE DIRECTIONS

Although it has taken a long time, it is now clear that purines and pyrimidines play pivotal roles in a variety of physiological activities in the gastrointestinal tract of mammals, including man. The most recent work has focused on the pathophysiological roles of purinergic signalling in the gut and I believe that the time is ripe for serious exploration of the therapeutic potential of purinergic compounds for a variety of gut disorders.

While there are some studies of perinatal development of purinergic signalling in the mammalian gut,[5] I believe that further studies should be encouraged, particularly concerning purinergic signalling in enteric stem cells involved in development and regeneration, with implications in paediatric and geriatric medicine.

CONFLICTS OF INTEREST

GB has declared no conflicts of interest.

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