

ATP and Sensory Transduction in the Enteric Nervous System

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ATP is a neurotransmitter in the central and peripheral nervous systems and is also involved in peripheral inflammation and transmission of the sensation of pain. Recently, the regulated release of ATP from non-neuronal sources has been shown to play a role in the activation of sensory nerve terminals. Within the enteric nervous system, which is present in the wall of the gastrointestinal tract, ATP plays three major roles. ATP acts as an inhibitory transmitter from the enteric motor neurons to the smooth muscle via P2Y receptors. ATP is released as an excitatory neurotransmitter between enteric interneurons and from the interneurons to the motor neurons via P2Y and P2X receptors. Finally, ATP may act as a sensory mediator, from epithelial sources to the intrinsic sensory nerve terminals. Thus, ATP participates in the transduction of sensory stimuli from the gut lumen and in the subsequent initiation and propagation of enteric reflexes. *NEUROSCIENTIST* 9(4):243–260, 2003. DOI: 10.1177/1073858403253768

KEY WORDS: Purines, Synaptic transmission, Sensory neurons, Sensory transduction, Gastrointestinal tract, Enteric nervous system

Just as “the wine in the bottell doth not quench thirst” (from *Jacula Prudentum*, George Herbert), food in the gut does not provide sustenance—it must first be digested and absorbed. Unfortunately, this is not as simple as opening a bottle of wine; the functions of the gastrointestinal (GI) tract are controlled by a complex interplay between local nerves and muscle, central innervation, and hormonal control.

The basis of GI function rests on its ability to convert ingested food into nutrients that can be absorbed and used by the body and protect it from environmental toxins and pathogens. Yet the nerve terminals of the sensory neurons that control these functions do not come into contact with the luminal contents. This review considers the role of the enteric nervous system (ENS) in sensing and acting on nutrients and toxins present in the intestinal lumen. The intestine may sense contents by using an intermediate step between the initial chemical or mechanical stimulus and the subsequent activation of sensory neurons in the wall of the intestine. In this proposed strategy, specialized epithelial cells sense changes in the lumen (e.g., nutrients, distortion of the epithelium) and pass this information on via the regulated release of

sensory mediators that excite the sensory nerve terminals directly. The role of 5-HT as a sensory mediator will be reviewed and the role of ATP as a sensory mediator introduced.

Structurally, ATP is a purine; thus, transmission involving ATP is called purinergic (Ralevic and Burnstock 1998). ATP acts at G-protein-coupled (metabotropic) P2Y receptors (for review, see von Kugelgen and Wetter 2000) and ligand-gated P2X receptors/ion channels (for review, see Khakh and others 2001; North 2002). There are three ways ATP may come into contact with these receptors: the regulated release of ATP from neuronal sources (i.e., as a neurotransmitter), the unregulated release from damaged or inflamed tissue, or the regulated release from nonneuronal sources. In the first instance, ATP and related purines are recognized as neurotransmitters in the CNS (e.g., Edwards and others 1992), peripheral nervous system (e.g., Evans and others 1992; Silinsky and others 1992), and ENS (reviewed in Galligan 2002). Recently, in the CNS, P2X receptors have also been found to have a role in long-term potentiation (Armstrong and others 2002; Pankratov and others 2002). In the second instance, the unregulated release of ATP occurs in the periphery from damaged tissue and is involved in peripheral inflammation and activation of cutaneous sensory nerve terminals; transmission of these nociceptive signals to the CNS may also involve purinergic neurotransmission from neurons at the level of the dorsal root ganglia (for review, see Burnstock and Wood 1996; Wood and Docherty 1997). In the final instance, also in the periphery, the regulated release of ATP from nonneuronal sources can activate sensory nerve terminals and, thus, transduce sensory stimuli. For example, in the carotid bodies, co-release of ATP and acetylcholine from the epithelially derived glomus cells

This work was supported by grant 114103 and fellowship 007703 from the National Health and Medical Research Council, Australia. Many personal and professional thanks to Dr Heather M. Young, Dr Joel C. Bornstein, and Tricia M. Wright for helpful discussions and insights in this article. Special thanks to Rebecca L. Monro and Dr Joel C. Bornstein for contributions to Figure 3 and Madeleine P. Stephens for contributions to Figure 4. This work was supported by grant 114103 and fellowship 007703 from the National Health and Medical Research Council, Australia.

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mediates the hypoxic (low blood pO_2) signal (Zhang and others 2000). More generally, Burnstock (2001) has hypothesized that stretch of tubular organs may release ATP from epithelial cells that then activates sensory nerve terminals.

Within the circuitry of the ENS, ATP is likely to play an excitatory role between enteric interneurons via P2Y receptors and from the descending interneurons to the inhibitory motor neurons via P2X receptors (for review, see Galligan 2002). Inhibitory transmission from the inhibitory motor neurons to the circular smooth muscle is also via P2Y receptors. Recent data from the guinea pig ileum suggest that ATP, released from epithelial cells lining the gut lumen, can act as a sensory mediator (Bertrand and Bornstein 2002). It can act directly through P2X receptors on the nerve terminal and through the release of serotonin from enterochromaffin (EC) cells.

The focus of this review is on recent developments in our understanding of the role of ATP in the function of the circuitry of the ENS as a signal between neurons and as a signal to the mucosal terminals of the intrinsic sensory neurons. The structure of the GI tract and the sensory innervation will be covered first, followed by a detailed examination of the properties of intrinsic sensory neurons from the guinea pig myenteric plexus. The nature of purinergic transmission within the ENS will be reviewed, including the localization of P2 receptors and the pharmacology and subunit composition of the P2X receptor (for an overview of the role of ATP and the P2X and P2Y receptors in the nervous system, see von Kugelgen and Wetter 2000; North 2002). Finally, the concept of sensory transduction in the GI tract and the role ATP may play will be explored, and some complex interactions between ATP and the release of 5-hydroxytryptamine or serotonin (5-HT) will be reviewed.

The GI Tract and the ENS

The GI tract is composed of many anatomically and functionally distinct regions (Gershon 1981; Furness and Costa 1987; Costa and others 2000). Except where noted, the examples used in this review will refer to guinea pig small intestine. Anatomically, there is the esophagus, stomach, the small intestine (bowel) with the closely related gall bladder and endocrine pancreas, the large intestine, and the rectum. These regions can be further subdivided; for example, the small intestine is composed of the duodenum; the jejunum; the proximal, middle, and distal ileum; and the terminal ileum. The function of the small intestine varies along its length, as do the types of luminal stimuli that each region comes into contact with. The movements of the small intestine in the presence of a nutrient is commonly a mixing or segmental motor pattern (e.g., in the canine jejunum, Schemann and Ehrlein 1986). Once a region of small intestine has absorbed a certain amount of nutrient, the lowered concentration presents a reduced stimulus, and the predominant movements are thought to switch to an anally directed propulsive, or peristaltic, motor pattern. These

reflexes are complex motor patterns that are made up of simpler reflexes such as the descending excitatory reflex, the descending inhibitory reflex, and the ascending excitatory reflex (for detailed descriptions of these reflexes, see Bornstein and others 2002b)

The Layers of the Gut Wall

The wall of the GI tract is composed of distinct, circumferentially orientated layers (Fig. 1). The mucosal epithelium contains cells responsible for secretion of electrolytes, protective mucous, and enzymes; for the absorption of nutrients and fluids; and for neuroimmune function (for review, see Cooke 1998). Among these epithelial cells are scattered enteroendocrine cells that contain a variety of neurotransmitter-like substances. Beneath the epithelial layer is the lamina propria and muscularis mucosa; this is the muscle layer responsible for the movements of the villi. Next is the submucosa, a matrix of collagen fibers that contains the blood and lymph vessels. Also at this level is the submucosal plexus, the first of two ganglionated nerve plexes that make up the ENS. It contains the neurons that innervate the epithelial cells (secretomotor nerves) and the blood vessels (vasodilator nerves) (for a complete list of neuronal types, see Box 1). The circumferentially orientated (circular) smooth muscle layer lies below this; it is responsible for the annular contractions that underlie the movement of the luminal contents. The next layer is the myenteric plexus, the second of the two ganglionated plexes and the one that contains the bulk of the interneurons (the circuitry of the ENS) as well as the inhibitory and excitatory motor neurons (Box 2). The submucosal and myenteric plexes each contain the cell bodies of sensory neurons that are intrinsic to the wall of the gut (see below). The final layers are the longitudinal muscle and the serosal membrane. Interspersed at several different levels are the interstitial cells of Cajal. These are specialized muscle cells that play a role in pace making or coordination of the smooth muscle, or facilitation of neuromuscular transmission (for review, see series starting with Ward and Sanders 2001).

Functional Classes of Enteric Neuron

There are different types of enteric neurons that vary in their shape, projection pattern, electrophysiological characteristics, and neurotransmitter content. Over the past 30 years, these neurons have been categorized into 18 functional classes (Furness and Costa 1987; Costa and others 1996; Brookes 2001). Each class of neuron can be uniquely identified by a combination of markers contained within them; this has been called a "chemical code" (Furness and Costa 1987). These markers may be, for example, neurotransmitters or their synthesizing enzymes, calcium-binding proteins, or vesicular transporters. The functional classes of enteric neurons are listed in Boxes 1 and 2; they include motor neurons, interneurons, and secretomotor and vasomotor neurons. Importantly, this work has led to the identification of a

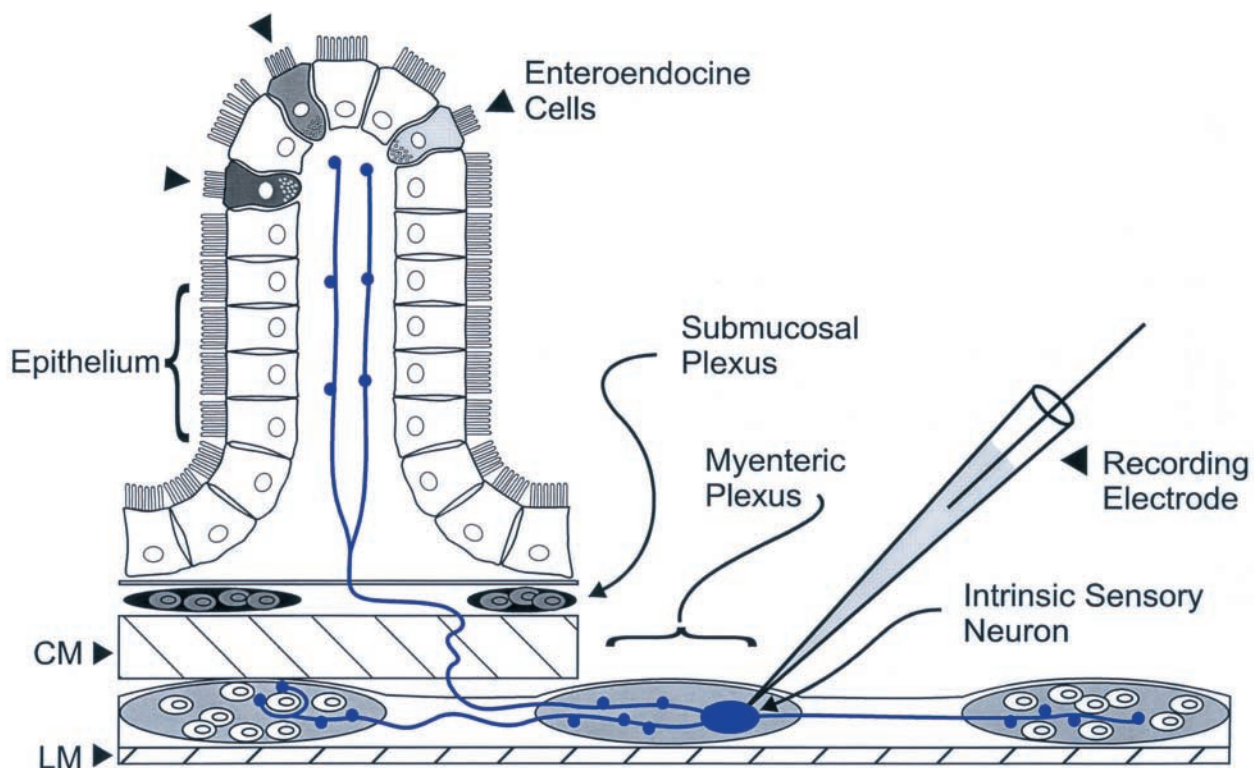


Fig. 1. The gut wall and the relation of the epithelium to the intrinsic sensory nerve terminal and cell body. The intestinal wall is divided into layers. From the bottom, these layers are the longitudinal muscle (LM); the myenteric plexus, which contains the cell bodies of motor neurons, interneurons, and a population of intrinsic sensory neurons—depicted; the circular muscle (CM); the submucosal plexus, with the cell bodies of the secretomotor and vasodilator neurons; and the mucosal epithelium, which contains the enteroendocrine cells—these are specialized epithelial cells that contain neuroactive substance in basally located secretory granules; several types are depicted. Note that the circular muscle, submucosal plexus, and epithelium have been removed from the right half of the preparation to allow an intracellular recording electrode to impale a myenteric sensory neuron. When the cell body of the intrinsic sensory neuron is close enough to the intact mucosa (<1 mm), there is a good chance that one or more of its projections still innervates the mucosa. Other projections innervate adjacent ganglia.

Box 1. Functional Classes of Neurons in the Submucosal Plexus of the Guinea Pig Ileum

Functional Class	Target	Chemical Code
Vasodilator	Blood vessels	VIP or NPY
Secretomotor	Epithelial cells	VIP or NPY
Sensory neurons	Other neurons, mucosa	ChAT, SP, calbindin, NeuN
Interneurons	Other neurons	Unknown

VIP = vasoactive intestinal peptide; NPY = neuropeptide Y; ChAT = choline acetyl transferase; SP = Substance P; NeuN = a protein found in the nucleus of all neurons and in the cytosol of the intrinsic sensory neurons. For review, see Furness (2000) and Brookes (2001).

class of sensory neuron that is intrinsic to the wall of the gut (see below).

Sensory Innervation of the Intestine

In this review, the first neuron in a reflex arc is defined as a sensory neuron. It may be purely sensory—that is, responding directly to a primary environmental stimulus such as stretch of the intestinal wall or an afferent—responding secondarily to activation of a sensor cell (for

more details, see Furness and others 1998). A major theme of this review is that many of the sensory nerve terminals in the intestine respond to mediators released from nonneuronal sensor cells.

It has been known for more than 100 years that the isolated intestine responds to the luminal environment; an increase in pressure in the lumen, a pinch of the mucosa, or a crystal of sodium chloride placed on the mucosa all evoked an immediate, neurally mediated motor response—this is a simple reflex arc (Furness and

Box 2. Function Classes of Neurons in the Myenteric Plexus of the Guinea Pig Ileum

Functional Class	Target(s)	Chemical Code
Sensory neurons	Other neurons, mucosa	SP, ChAT, calbindin, NeuN
Descending interneurons	Other neurons	SOM or 5-HT or VIP
(+) CM motor neurons	CM	ChAT, SP
(-) CM motor neurons	CM	NOS, VIP
(+) LM motor neurons	LM	ChAT, SP, calretinin
Ascending interneurons	Other neurons	ChAT, SP, calretinin
Vasodilator	Blood vessels	VIP or NPY
Secretomotor	Epithelial cells	VIP or NPY

The chemical codes, in brief, of the major functional classes of neurons. SP = substance P; ChAT = choline acetyl transferase; calbindin = a calcium-binding protein; NeuN = a protein found in the nucleus of all neurons and in the cytosol of the intrinsic sensory neurons; SOM = somatostatin; 5-HT = 5-hydroxytryptamine or serotonin; VIP = vasoactive intestinal peptide; (+) = excitatory; CM = circular smooth muscle; (-) = inhibitory; NOS = nitric oxide synthase; LM = longitudinal smooth muscle; ascending = orally directed projections; descending = anally directed projections; NPY = neuropeptide Y. For review, see Costa and others (1996) and Brookes (2001).

Costa 1987). It is only in the past 30 years that the neuronal circuitry underlying these enteric reflexes has been elucidated. It is now known that the intestine is capable of generating many simple reflexes as well as complex motor patterns even when fully isolated from the CNS (Bornstein and others 2002b). These reflexes are initiated by the complex sensory innervation within the GI tract, of which there are two types: sensory innervation originating from neurons with cell bodies outside the gut wall is extrinsic, whereas innervation originating from neurons with cell bodies within the gut wall is intrinsic. Both sets of sensory nerves are sensitive to nutritive and noxious stimuli, but they mostly function independently of one another.

Extrinsic Sensory Innervation

Extrinsic sensory nerves have cell bodies in dorsal root ganglia and nodose ganglia; these fibers run predominantly in the spinal and vagal tracts, respectively. They can be referred to as extrinsic primary afferent neurons (EPANs) or, more simply, as extrinsic sensory neurons. The spinal afferents are thought to convey mainly nociceptive stimuli to the CNS and innervate the length of GI tract more or less equally. The vagal afferents are thought to convey mainly those feelings from the intestine that reach the level of consciousness, such as satiety (Grundy and Scratcherd 1997). They do not innervate the GI tract equally. Instead, there is a dense innervation of the esophagus and stomach with a progressively less dense innervation of the small and large intestines (for review, see series starting with Powley and Phillips 2002). Their chemical and mechano-sensitivity have been studied (e.g., Berthoud and others 2001; Page and others 2002) and reviewed (e.g., Kirkup and others 2001; Grundy 2002). Importantly, the sites at which transduction of mechanical signals take place in the esophagus and stomach have been identified as specialized ramifications of the terminals called intraganglionic laminar

endings (Zagorodnyuk and Brookes 2000; Zagorodnyuk and others 2001). The vagal and spinal innervation participates in many enteric reflexes, even when decentralized, by way of axon reflexes. In the colon, in particular, some basic reflexes appear to be mediated wholly by this innervation (e.g., Grider and Jin 1994).

Intrinsic Sensory Innervation

Intrinsic sensory nerves have cell bodies in the myenteric and submucosal plexes (Fig. 2). They have projections to the mucosa and other enteric ganglia and are referred to as intrinsic primary afferent neurons (IPANs) (Furness and others 1998) or, in this review, as the intrinsic sensory neurons. The intrinsic sensory neurons were first identified in a preparation of guinea pig ileum developed by Kunze and colleagues (1995). This preparation enabled intracellular recordings to be made from the neurons located in the myenteric plexus about 1 mm circumferential from a region of undisturbed mucosa. In this situation, about half the intrinsic sensory neurons were left with an intact projection to the mucosa, whereas those sensory neuron with cell bodies more than 1 mm away rarely projected this far. These projections ramify within the lamina propria below the mucosal epithelium, and their receptive field can be mapped physiologically with either a small, localized application of chemical stimulant to the mucosa or by focal mechanical or electrical stimulation.

Sensory Modalities of Intrinsic Sensory Neurons

Intrinsic sensory neurons have only been definitively identified and characterized in the guinea pig ileum (see Furness and others 1998). There are three basic modalities to which they respond: chemical or mechanical stimulation of the mucosa, or distension of the gut wall. Mucosal chemosensory neurons have been identified that have cell bodies in the myenteric plexus that are sen-

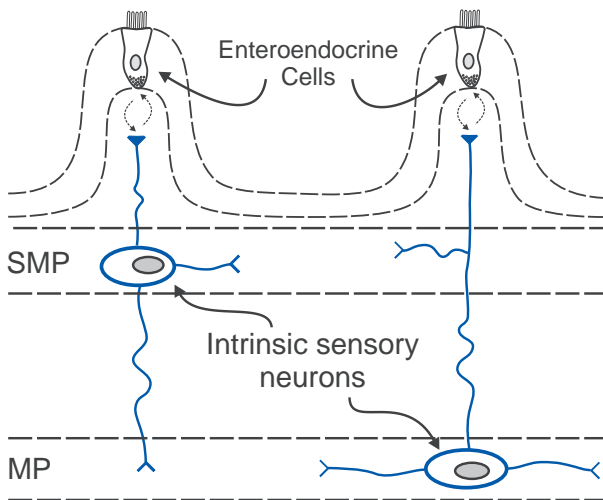


Fig. 2. Sensory innervation of the intestine. A side view of the wall of the gut showing the connections from the intrinsic sensory neurons located in the submucosal plexus (SMP) and myenteric plexus (MP). The intrinsic sensory neurons are multipolar, and their terminals project to the submucosal plexus, myenteric plexus, and the mucosal epithelium where they come into contact with the enteroendocrine cells. All the projections of the intrinsic sensory neurons can conduct action potentials, and all are specialized for release of neurotransmitter; thus, all can be labeled axons.

sitive to acid at a low pH (1–3) (Kunze and others 1995), acid at a more physiological pH (3–5), to a high (basic) pH (9–11), and to the short-chain fatty acid acetate at a neutral pH (7) (Bertrand and others 1997). A large proportion of these chemosensory neurons are also sensitive to the putative sensory mediators, 5-HT (Bertrand and others 1997, 2000) and ATP (see below, and Bertrand and Bornstein 2000, 2002). Intrinsic sensory neurons that are sensitive to small areas (<1 mm²) of distortion of the mucosal epithelium have been identified in the submucosal plexus. The evidence suggests that the mechanical stimuli selectively causes release of 5-HT from EC cells (a subtype of the enteroendocrine cells; see below) that then activates the sensory nerve terminals (Kirchgessner and others 1996; Pan and Gershon 2000). Myenteric sensory neurons are generally not sensitive to distortion of small areas (<1 mm²) of mucosa (Kunze and others 1995; Bertrand and others 1997); however, the presence of L-type calcium channel blockers may have contributed to this negative result. L-type calcium channel blockers are commonly used to paralyze the smooth muscle during intracellular recordings and are known to reduce the release of 5-HT from EC cells (see below, and Racké and Schwörer 1993). Intrinsic sensory neurons in the ileum that are sensitive to distension of the gut wall are located in the myenteric plexus (Kunze and others 1998). When the smooth muscle is paralyzed, their activation is inhibited (Kunze and others 1999), suggesting that they are sensitive to stretch of the muscle and not a change in length. There is also a population of length sensitive intrinsic sensory neurons in the colon (Bywater 1994), but their identity is as yet uncertain (Spencer and others 2002).

Morphology and Projection Pattern of Intrinsic Sensory Neurons

The anatomy of the intrinsic sensory neurons in the guinea pig ileum is distinct; their shape and projection patterns fit with that described by Dogiel (1899) as type-II cells (Furness and others 1998) (Fig. 1). Dogiel type-II neurons account for 25% to 35% of all myenteric neurons in the guinea pig ileum (Costa and others 1996, 2002). Compared to other enteric neurons, they have a large cell body (30–60 μm diameter) with no dendrites (i.e., smooth bodied, but see below) and are located in the myenteric and submucosal plexes. They are multipolar and have projections that run to the mucosal epithelium, between the myenteric plexus and the submucosal plexus, and to many other cells within the ganglia in which the cell body is located and to other closely located ganglia (Pompolo and Furness 1988) (also see Fig. 2)—the projections are predominately in the circumferential directions. All of these projections can conduct action potentials (APs) (Hendriks and others 1990), and all contain varicosities specialized for release of neurotransmitter; thus, all can be labeled as axons. Synaptic contacts on the Dogiel type-II neurons are predominately on the cell body (Pompolo and Furness 1988). In addition to these local projections, studies of descending inhibitory reflexes have shown that some distension-sensitive intrinsic sensory neurons must have long anal projections (Johnson and others 1996, 1998; Bian and others 2000, 2003). Dogiel type-II neurons with a single long descending projection have been identified and account for 10% to 20% of the intrinsic sensory neurons; they also appear to have more numerous local projections, giving them a dendritic appearance (Bornstein and others 1991; Brookes and others 1995). In the small intestine, projections from Dogiel type-II neurons to the mucosa innervate the full length of the villi (Li and Furness 1998). The anatomical field innervated by a single neuron has been estimated as 1 mm² using retrograde dye techniques (Song and others 1991) and > 6 mm² using electrical stimulation of the mucosa (Bertrand and others 1998).

Electrophysiological Properties of Intrinsic Sensory Neurons

In the guinea pig ileum, intrinsic sensory neurons have AH-type electrophysiological characteristics (i.e., a long-lasting afterhyperpolarizing potential [AHP] following the action potential [AP]; Hirst and others 1974, 1985b). The AHP is thought to greatly reduce the excitability of the sensory neuron cell body. The basis of the AHP is calcium entry during the AP that opens a calcium-activated potassium conductance (Hirst and others 1974, 1985a; Vogalis and others 2002). Intrinsic sensory neurons receive fast synaptic input only infrequently but have been found to possess many of the receptors that mediate such input (e.g., nicotinic ACh, 5-HT₃, GABA_A) (Wade and others 1991; Bertrand and Galligan 1992; Bornstein and others 1994; Zhou and Galligan 1998). A recent addition to this complement of

receptors is the finding that P2X receptors are on many intrinsic sensory neuron cell bodies (see below; Bertrand and Bornstein 2002).

Slow Synaptic Input to Intrinsic Sensory Neurons

Ileal intrinsic sensory neurons have prominent slow synaptic input (i.e., slow excitatory postsynaptic potential [EPSP]). The source of these slow EPSPs is mainly from other intrinsic sensory neurons (Kunze and others 1993); thus, as a group, the sensory neurons form a self-reinforcing network. Because they have predominately circumferentially orientated projections, this probably allows for the coordination of reflexes such that the full circumference of a region is involved (Thomas and others 2000) and may underlie the initiation or propagation of some reflexes (e.g., Thornton and Bornstein 2002). The slow EPSP is primarily mediated by substance P or a related tachykinin acting at neurokinin receptors (NK₁ and possibly NK₂) (Bertrand and Galligan 1994; Johnson and others 1998; Alex and others 2001). The sensory neurons have, however, many G-protein coupled receptors that when activated produce a slow EPSP-like depolarization (e.g., North and Tokimasa 1983; Palmer and others 1987; Bertrand and Galligan 1995). In particular, receptors for the gut sensory mediators/transmitters 5-HT and cholecystikinin (CCK) have been demonstrated (Mawe and others 1986; Palmer and others 1987), as have receptors for the inflammatory mediators prostaglandin PGE₂ and histamine (Nemeth and others 1984; Dekkers and others 1997) and for ATP acting through inhibitory P2Y receptors (Katayama and Morita 1989).

Long-Term Changes in Intrinsic Sensory Neuron Properties

It is now known that some of the properties of the intrinsic sensory neurons are not fixed but vary over time. For example, the AHP, which is thought to greatly reduce the excitability of the sensory neuron cell body, can itself be reduced (a reduction in amplitude and duration) during a slow EPSP, following prolonged stimulation or during inflammation (Morita and others 1982; Palmer and others 1998; Clerc and others 1999; Linden and others 2003). Similarly, the amplitude of fast EPSPs and frequency with which they are seen is increased in sensory neurons from inflamed tissue (Palmer and others 1998; Linden and others 2003). Controlling these changes in the excitability of the intrinsic sensory neuron may be an important therapeutic target (e.g., Clerc and others 2002).

Chemical Code of Intrinsic Sensory Neurons

In the guinea pig ileum, the chemical code for the intrinsic sensory neurons is relatively simple. All the sensory neurons contain choline acetyl transferase (ChAT, the ACh synthesizing enzyme) and are, thus, cholinergic. In addition, up to 80% contain the neuropeptide substance P, and most also contain the neuropeptide neuromedin U. There are also markers that distinguish the sensory neu-

rons from the interneurons and motor neurons. Only the sensory neurons contain the calcium-binding protein calbindin (around 80%), and in their cytoplasm, most sensory neurons are reactive for the nuclear marker NeuN (Costa and others 2002; but also see Castelucci and others 2002). Note that all myenteric neurons contain NeuN in the nucleus, and this has been used in studies to count total numbers of neurons (e.g., Costa and others 2002).

Are Intrinsic Sensory Neurons Modality Selective?

The intrinsic sensory neurons have been characterized in terms of sensory modality, anatomy, electrophysiological properties, and chemical code. There are those in the submucosal plexus and those in the myenteric plexus (e.g., Kirchgessner and others 1992; Kunze and others 1995). Furthermore, in the myenteric plexus, there are those that have a long descending projection with dendritic morphology and those that do not (Bornstein and others 1991; Brookes and others 1995). Based on chemical coding, there are overlapping populations of intrinsic sensory neurons (Costa and others 2002). Sensory neurons with different chemical codes appear, however, electrophysiologically and morphologically much the same (Furness and others 1998), and the same is true for the other properties. Thus, at this stage, there is no clear correlation that cuts across anatomy, electrophysiology, and chemical code such that specific properties are matched with specific modalities.

To understand how the sensory neurons control enteric reflexes, we must know how they encode the sensory information. Thus, whether there are modality-specific populations of intrinsic sensory neuron and whether any other properties correlate with the modality is an important question. Sensory information could, in the simplest arrangement, be encoded in two ways, although these ways need not be exclusive of one another. First, the intrinsic sensory neurons could consist of separate populations with different modality specificity and different connections with other neurons in the enteric circuitry. That the submucosal sensory neurons appear to be sensitive to distortion of the mucosa whereas the myenteric sensory neurons appear more sensitive to chemical stimuli would support this idea.

Second, all intrinsic sensory neurons could be composed of a single population with similar connections but with the ability to generate different patterns of AP discharge for different sensory stimuli. Evidence for this is that single myenteric sensory neurons respond to a range of chemical, nutrient, and, more rarely, mechanical stimuli applied to the mucosa (Bertrand and others 1997). Many also respond to mucosally applied 5-HT (Bertrand and others 1997; Bertrand and others 2000) or ATP (Bertrand and Bornstein 2000, 2002). In addition, all myenteric sensory neurons project to the mucosa (Song and others 1994), suggesting that some distension-sensitive myenteric sensory neurons must also be sensitive to changes within the lumen. Together, these observations would seem to rule out separate populations of

intrinsic sensory neurons with different modalities and suggest that there is a continuum.

ATP and P2 Receptors in the ENS

ATP acts at two main classes of P2 receptors: the P2X receptor, which is a ligand-gated ion channel, and the P2Y receptors, which are G-protein-coupled (metabotropic) receptors. For a definitive review of both P2X and P2Y receptors, see Ralevic and Burnstock (1998).

Properties of P2X Receptors

There are seven subtypes of P2X receptors found in adult tissue, P2X₁₋₇, and each subunit has two membrane-spanning domains (Humphrey and others 1998; Khakh and others 2001; North 2002). Between two and six subunits might make up a functional receptor/channel complex, but the most likely number is three subunits. The receptor can be composed of the same (homomeric) or different (heteromeric) subunits. All P2X subtypes can form homomers, except the P2X₆, which only mixes with different subtypes, whereas all P2X subtypes can form heteromers, except the P2X₇ receptors, which do not mix with the other subtypes. Many of the homomeric and heteromeric receptors have different pharmacologies. For example, ATP is a ligand at all of these receptors, but α,β -methylene-ATP or 2-methylthio-ATP may be more potent at individual receptors. These differences, coupled with the different biophysical properties some channels display, can be used to identify the composition of receptors in native tissue (e.g., Torres and others 1999) (see below).

Properties of P2Y Receptors

There are at least eight subtypes of P2Y receptors with more likely awaiting discovery (Harden and others 1998; von Kugelgen and Wetter 2000). The five main subtypes of receptors are the P2Y_{1,2,4,6,11}; all couple to activation of phospholipase C, and in addition, P2Y₁₁ couples positively to adenylate cyclase. ATP is a ligand at all of these receptors, but uridine 5'-triphosphate, uridine 5'-diphosphate, or ADP may be more potent and can be used pharmacologically to distinguish between them (von Kugelgen and Wetter 2000).

Determination of P2 Receptor Subtypes in the ENS

Although the presence of P2 receptors in many systems is clearly established, the particular subtypes of P2X or P2Y receptors that are used have not been fully determined. This has been due to the general lack of P2 receptor subtype selective antagonists. The two most commonly used P2 antagonists, pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) and suramin, seemingly bind at random to the different subtypes of P2X and P2Y receptors (see Ralevic and Burnstock 1998). Attempts to identify particular P2 receptors center around using pharmacological and biophysical prop-

erties that are first determined from recombinant receptors in heterologous expression systems (e.g., *Xenopus* oocytes). For example, the rank order of potency of ATP and ATP analogs can be used to identify a receptor, or more likely to provide a short list of potential receptors, that might be present in the native tissue. Similarly, the biophysical properties of the receptors can be exploited to aid in identification. For example, P2X₂ receptors pass more current when the extracellular solution is slightly acidic, whereas P2X₂ and P2X₄ receptors are potentiated in the presence of zinc ions. Also, P2X₁ and P2X₃ receptors are distinguished by their rapid desensitization. Finally, although PPADS and suramin discriminate poorly between receptors, they can be used in conjunction with the techniques above to provide a definitive receptor classification.

P2X and P2Y Receptors in the Gut

A variety of studies have shown that there are P2X and P2Y receptors on nerves and muscle in the GI tract. On the longitudinal smooth muscle, there are excitatory P2X receptors, whereas on the circular smooth muscle, there are inhibitory P2Y receptors. On the neurons, P2X and P2Y receptors exist both pre- and postsynaptically and have mainly excitatory roles. The physiological role of many of these receptors in enteric reflexes is unclear, but the characterization of the receptor locations and functions has begun to address this. Three main approaches have been used to identify effects caused by ATP or mediated by P2 receptors: localization of receptors (either mRNA or protein), localization of ATP itself, or functional studies where exogenous application of ATP is used to evoke a response or the application of a P2 receptor antagonist is used to block a response to nonphysiological (e.g., electrical activation of nerve fibers) or physiological (e.g., distention of the gut wall) stimuli.

Localization of P2X Receptors in the ENS

In the guinea pig ileum, P2X₂ receptors have been localized in the myenteric plexus to populations of nitric oxide synthase (NOS)-positive interneurons or motor neurons and on intrinsic sensory neurons (Castelucci and others 2002). Ninety percent of sensory neurons stained for the P2X₂ receptor, with one-third of these staining strongly and the rest staining weakly. In the mouse intestine, Giaroni and others (2002) localized P2X₂ receptor mRNA to a much smaller population of myenteric neurons of unknown functional class. In the guinea pig, Poole and others (2002) and Van Nassauw and others (2002) found P2X₃ receptors on a variety of myenteric neurons, including some NOS-positive neurons and ascending interneurons or longitudinal muscle motor neurons, but not on sensory neurons. Thus, it appears that the intrinsic sensory neurons do not have P2X₃ receptors, and most only stain weakly for the P2X₂ receptor. These data are at odds with the numbers of sensory neurons that respond to exogenous application of

ATP (see below; Bertrand and Bornstein 2002), suggesting that other P2X receptors are involved.

Localization of ATP in the ENS

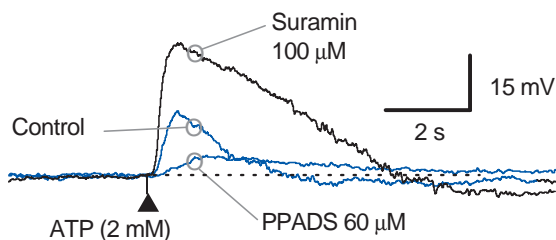
Localization of ATP itself is more difficult than localization of the receptors (Ralevic and Burnstock 1998). Unlike classical transmitters or neuropeptides, purines are difficult to localize using standard histochemical techniques. Quinacrine staining has been used by some groups to visualize the high concentrations of ATP in vesicles (e.g., Brouns and others 2000), although the specificity of this technique has never been 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 neurons that might release ATP cannot be found directly.

Functional Evidence for Purinergic Transmission in the ENS

In electrophysiological studies in guinea pig ileum, the P2 receptor antagonists PPADS or suramin block hexamethonium-resistant fast EPSPs (Galligan and Bertrand 1994; Johnson and others 1999; reviewed in Galligan 2002); similar data were found in the distal colon (LePard and others 1997; Nurgali and others 2003) and in the duodenum, taenia coli, and proximal colon, but not gastric corpus (LePard and others 1997). Together, these data suggest that endogenous ATP acting at P2X receptors mediates fast EPSPs between many myenteric neurons. Evidence from combined electrophysiological and lesion studies has shown that some of these P2X receptors are in descending pathways (LePard and Galligan 1999). Recent data from the guinea pig submucosal plexus show that P2X-mediated fast EPSPs are common (Figure 3B, Monro and others 2002b, 2003), as are slow EPSPs (Hu and others 2002). One thing these studies have in common is that they used electrical stimulation to evoke the fast and slow EPSPs. Electrical stimulation excites all neural pathways, so few conclusions can be drawn about the source of these fast EPSPs or their physiological role.

Studies in guinea pig ileum have used physiological stimuli in an attempt to find a role for the purinergic EPSPs. One study examined the role of P2X receptors in descending excitatory reflexes evoked by stroking the mucosal epithelium (Spencer and others 2000) and concluded that these receptors have a role in transmission between interneurons in this pathway. In support of this, Monro and others (2002a), recording changes in muscle length, found that transmission in the descending excitatory pathway could be blocked with the P2 receptor antagonist PPADS. In a study on the descending inhibitory pathways, Bian and others (2000), using a partitioned organ bath and intracellular recording from circular smooth muscle, found that transmission from interneurons to inhibitory motor neurons was largely via P2X receptors. More recently, Thornton and Bornstein,

A. Intrinsic sensory neuron



B. Secretomotor neurons

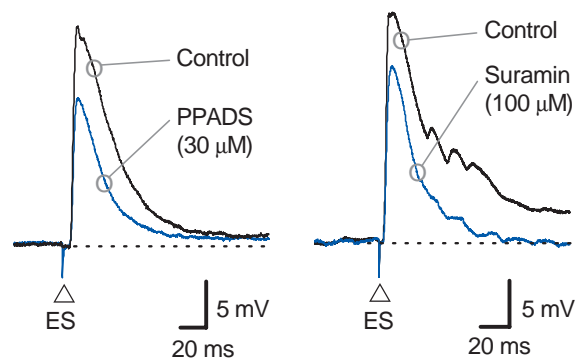


Fig. 3. P2X receptors on intrinsic sensory neurons and secretomotor neurons. Representative voltage recording from an intrinsic sensory neuron in the myenteric plexus (A) and two secretomotor neurons from the submucosal plexus (B). The dotted lines indicate resting membrane potential (RMP). A, ATP was applied to the cell body of an intrinsic sensory neuron and evoked a short latency depolarization—tetrodotoxin was present to block sodium dependent action potentials. During superfusion with pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) (60 μ M), the ATP-evoked depolarization was blocked, whereas in the presence of suramin (100 μ M), it was potentiated (RMP: control = -67 mV, PPADS = -75 mV, suramin = -77 mV). B, Electrical stimulation (ES) of intraganglionic fiber tracts with a single pulse (0.5 ms duration) evoked a fast excitatory postsynaptic potential (EPSP) that was not completely blocked by a nicotinic receptor antagonist. The fast EPSP was partially reduced by PPADS (30 μ M) (left) and by suramin (100 μ M) (right) suggesting that the noncholinergic component is due to P2X receptors (RMP was -80 mV for all conditions). Panel A was adapted from Bertrand and Bornstein (2002), copyright by Society for Neuroscience.

recording intracellularly from myenteric neurons, found that distension-evoked slow EPSPs were blocked by PPADS (Bornstein and others 2002a). These slow EPSPs only occurred in NOS-positive descending interneurons (Thornton and Bornstein 2002). These data are supported by work in mouse demonstrating that P2Y₁ receptors are on NOS-positive myenteric neurons (Giaroni and others 2002) and by work in guinea pig demonstrating that exogenous application of ATP evokes a P2Y receptor mediated depolarization in many myenteric interneurons and motor neurons (Katayama and Morita 1989).

Unfortunately, the specific subtypes of P2 receptors used in enteric reflexes are mostly unidentified, either because the pharmacology is incomplete for the particular functional response or because the pharmacological or biophysical properties do not match with what has been reported in the literature. One exception is the evidence that the P2X receptors on the interneurons and/or motor neurons have a pharmacology that matches the P2X₂ receptors (Galligan 2002); this is supported by the immunohistochemical localization of P2X₂ receptors to many of these neurons (Castelucci and others 2002).

P2 Receptors on Enteric Smooth Muscle

Neuromuscular transmission also involves P2X and P2Y receptors. P2X receptors are found functionally on the circular muscle of human jejunum (Xue and others 1999), and recently, excitatory P2X receptors have been found on smooth muscle in mouse colon (Giaroni and others 2002). P2Y receptors are on circular muscle as shown by work demonstrating a large component of inhibitory transmission to circular muscle is via ATP in conjunction with nitric oxide and peptides like vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide (e.g., Crist and others 1992; He and Goyal 1993). This is supported by more recent work in guinea pig ileum (Bian and others 2000) and in mouse colon (Spencer and others 1998). Thus, some inhibitory motor neurons must be purinergic. In guinea pig, all inhibitory motor neurons can be identified by the presence of both NOS and vasoactive intestinal peptide (Costa and others 1996). Interestingly, the class of descending interneuron, implicated above as having a role in purinergic transmission in descending reflex pathways, is also NOS positive (Bian and others 2000; Bornstein and others 2002a). These data, taken together, suggest that many of the purinergic nerves in the gut also release nitric oxide as a transmitter or vice versa.

P2 Receptors on the Intrinsic Sensory Neurons

Recently, we have found in the guinea pig ileum that the majority of intrinsic sensory neuron cell bodies respond to exogenous application of ATP with a fast, P2X receptor-like response (Bertrand and Bornstein 2002). ATP (1–2 mM in a pressure pipette), applied to the cell body, evoked a large depolarization in 75% of the sensory neurons tested. This depolarization was potentiated by the P2 receptor antagonist suramin (100 μ M in the bath) and blocked by PPADS (60 μ M in the bath) (Fig. 3A). This is in contrast to ATP-mediated fast EPSPs recorded in other neurons from myenteric (reviewed in Galligan 2002) or submucosal ganglia (Monro and others 2002b) (Fig. 3B) that are blocked by either PPADS or suramin. The receptors on the myenteric sensory neuron cell bodies are activated by ATP, ATP- γ -S, and 2-methylthio-ATP (2-Me-S-ATP) at a high potency, but not by α , β -methylene-ATP (Fig. 4). The receptor subtype expressed on the cell body appears to be the same or similar to that expressed on the sensory nerve terminals in the mucosa

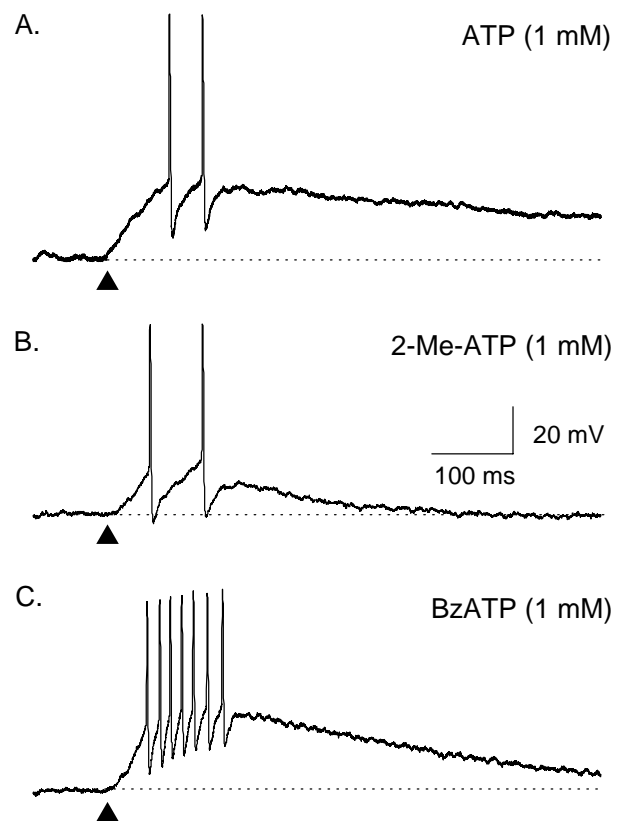


Fig. 4. ATP-evoked depolarizations of intrinsic sensory neurons. Representative voltage recording from three intrinsic sensory neurons in the myenteric plexus. The scale bars in *B* apply to all traces. The dotted lines indicate resting membrane potential (RMP). *A*. ATP (1 mM, 100 ms pressure application to the mucosa, applied at the filled triangle) was applied to the cell body of an intrinsic sensory neuron and evoked a short latency depolarization with two action potentials—tetrodotoxin was not present. Analogs of ATP, 2-methylthio-ATP (2-Me-S-ATP, 1 mM) and, 2'- and 3'-O-(4-benzoylbenzoyl)-ATP (BzATP, 1 mM) (RMP: ATP = -63 mV, 2-Me-S-ATP = -46 mV, BzATP = -59 mV).

(see below). Barajas-Lopez and others (1996), working with cultured myenteric neurons of unknown functional class (although they were likely to be sensory neurons), demonstrated a fast P2X-mediated current with several similarities in pharmacology. For example, α , β -methylene ATP was a weak agonist, whereas 2-Me-S-ATP was a strong agonist in both studies, and in both, the ATP-evoked depolarization was potentiated, rather than blocked, by suramin.

The pharmacology of the P2X receptor deduced from these studies is not well correlated with the pharmacologies of known P2X receptor subtypes. The lack of a correlation could be because a favored model for GI research, the guinea pig, appears to have P2X receptors with atypical pharmacology. For example, recent evidence suggests that guinea pig pelvic neurons express a P2X receptor with characteristics not seen in rat pelvic neurons (Dunn and others 2001; Zhong and others 2001).

P2Y receptors are also present on intrinsic sensory neurons but appear to mediate a hyperpolarization of the membrane due to an opening of the calcium-activated

potassium conductance (Katayama and Morita 1989). Christofi and others (1997), looking at cultured myenteric neurons (including some that were calbindin positive and, thus, likely to be intrinsic sensory neurons), have shown that there is a rise in intracellular calcium associated with application of ATP suggesting that ATP is causing calcium release from intracellular stores that then activates the potassium conductance.

P2X₇ Receptors in the ENS

P2X₇ receptors are generally thought to participate in apoptotic pathways via formation of a large cation permeable pore (North 2002) and are found on macrophages and other immune cells (Chow and others 1997). Newer studies, such as Kim and others (2001a), have shown that P2X₇ receptors in the nervous system cannot form pores but can operate as normal P2X receptors (albeit with some unusual second messenger coupling—see Armstrong and others 2002). Hu and colleagues (2001) found that cultured myenteric neurons (including some that were probably intrinsic sensory neurons) were depolarized by the potent, but nonselective, P2X₇ receptor agonist BzATP. BzATP binds with high affinity to P2X₁, P2X₂, and P2X₇ (Khakh and others 2001). The BzATP response was blocked by the selective P2X₇ receptor antagonist, oxidized ATP (Dell'Antonio and others 2002). In support of this, our preliminary data show that BzATP depolarizes identified intrinsic sensory neurons in the guinea pig myenteric plexus (Fig. 4). As P2X₇ receptors have not been well characterized pharmacologically, it is possible that some of the atypical pharmacology of the sensory neuron P2X receptors seen in previous studies is due to a population of other receptors and a population of P2X₇ receptors.

Sensory Mechanisms in the ENS

Nutritive or noxious stimuli in the lumen of the intestine do not appear to interact directly with sensory nerve terminals. Interaction is prevented, in the undamaged intestine, by the epithelial cells lining the lumen. In vitro, the epithelial cells are disrupted, and solutions may act directly on the nerve terminal within (e.g., see Fig. 1). This has been used to facilitate investigations into the nerve terminal receptor complement (see below). Under physiological conditions, however, a process of transduction must occur in which epithelial cells, or specialized cells of epithelial origin, sense the primary stimulus (e.g., distortion or chemicals) and release neuroactive substances on to nerve terminals in the underlying lamina propria (for review, see Furness and others 1999). This process of transforming a physiological stimulus into the firing of neurons is called sensory transduction.

Epithelial Cells

The epithelial cells lining the lumen have a life span of only a few days. During this time, the cells migrate from the crypts, where they are formed by dividing stem cells, to the surface epithelium, where they eventually undergo

apoptosis and are sloughed off into the lumen. The nerve terminals with which they make contact are small diameter, unspecialized endings from the intrinsic sensory neurons in the submucosal and myenteric plexes. Because of the constant migration, the nerve terminals appear to make only very few specialized contacts with the epithelial cells.

Enteroendocrine Cells

In the intestine, the enteroendocrine cells appear to function as sensors in much the same way as do other chemoreceptors in the carotid and aortic bodies or the neuroepithelial bodies (e.g., Gonzalez and others 1995; O'Kelly and others 1998). The enteroendocrine cells are specialized epithelial cells with microvilli on their luminal side and secretory granules located basally that can be released into the subepithelial (lamina propria) space. The contents of these granules may function as sensory mediators. Enteroendocrine cells contain many putative sensory mediators including: 5-HT (from the EC cells), cholecystokinin, somatostatin (from the D cells), neuropeptide Y, γ -aminobutyric acid (GABA), and ATP (Table 1). Some sensory mediators may be stored together in the same population of enteroendocrine cells, whereas others like 5-HT and CCK are stored in distinct subpopulations of enteroendocrine cells.

Transduction by Epithelial Cells

Sensory mediators are released by the epithelial cells during nutritive or noxious stimuli, but we know relatively little about the molecular basis for how the epithelial cells sense the primary stimulus and transduce it into release of a mediator. There have been a few studies that have attempted to equate taste (gustatory) mechanisms with intestinal chemoreception. For example, the gustatory G protein, gustducin, has been localized to intestinal brush cells. The brush cells have an unknown function but bear some morphological resemblance to the taste receptors in the tongue (reviewed in Hofer and others 1998). In this same vein, the newly discovered bitter taste receptor family (T2R) has been localized to epithelial cells, including some enteroendocrine cells, in rat stomach and duodenum (Wu and others 2002).

5-HT Release from EC cells

Of the sensory mediators investigated, only the release of 5-HT from the EC cell has been examined in detail. 5-HT has been hypothesized for almost 50 years to underlie some enteric reflexes (Bülbring and Crema 1958; for review, see Gershon 1991). 5-HT is stored in EC cells and has been implicated in pressure transduction from the lumen (Bülbring and Crema 1959; Kirchgessner and others 1992; Kim and others 2001b) as well as some nutritive stimuli (although not for lipids) (e.g., Kim and others 2001c). In general, the release of 5-HT has been found to be directly modulated by ligand-gated and G-protein-coupled receptors, as well as by mechanical influences (for review, see Racké and others 1996). 5-HT

Table 1. Types of Epithelial Cells in the Intestine

	Function	Granules	Sensory Mediators
Enteroendocrine	Hormone secretion		
EC cells		Basal	5-HT
D cells		Basal	Somatostatin
CCK cells		Basal	CCK
L cells		Basal	GLP
Paneth cells	Enzyme secretion	Apical	
Goblet cells	Mucous secretion	Apical	
Enterocyte	Absorption	Apical	ATP
Brush cell	Unknown	Apical	NO
Mast	Immune	Basal	Histamine, ATP?, 5-HT*

* Some species such as rat have 5-HT in mast cells.

EC = Enterochromaffin; 5-HT = 5-hydroxytryptamine or serotonin; CCK = cholecystokinin; GLP = glucagon-like-peptide; NO = nitric oxide. Note that other immune-related cells, such as M cells and cells that make up the Peyer's patches, are not included.

is released via a calcium-dependent process; however, in a study by Racké and Schwörer (1993), only 70% of 5-HT overflow was blocked in a high Mg^{++} , low Ca^{++} solution, whereas nifedipine, the L-type calcium channel blocker, reduced 5-HT overflow by only 50%; addition of an N-type calcium channel blocker was not additive. In addition, activation of muscarinic receptors also induced 5-HT release in the absence of external calcium, implicating a role for calcium from internal stores (Racké and others 1996). The actions of released 5-HT are thought to be terminated by the selective serotonin transporter (Wade and others 1996; Chen and others 1998).

Sensory Nerve Terminals Activated by 5-HT

It has been suggested that the nerve terminals of both the intrinsic and the extrinsic sensory neurons are activated by exogenously released 5-HT (for recent review, see Kirkup and others 2001). There is good evidence that 5-HT released from EC cells can act through 5-HT₃ receptors to excite the mucosal terminals of the myenteric intrinsic sensory neurons (Bertrand and others 2000) or the vagal extrinsic sensory neurons (Hillsley and others 1998) and can act through 5-HT_{1p} receptors to excite the terminals of the submucosal intrinsic sensory neurons (Pan and Gershon 2000). However, 5-HT is unlikely to be the only sensory mediator acting on the mucosal terminals of the sensory neurons as depletion of 5-HT from the mucosa, or addition of 5-HT receptor antagonists, does not block all of the many reflex actions of the intestine (e.g., Gershon 1991; Sanger 1996). It is more likely that several substances are acting in concert to encode the changing luminal environment (Kirkup and others 2001); one such substance may be ATP.

The Role of ATP in Sensory Transduction in the GI Tract

Burnstock (2001) has hypothesized that stretch stimuli in tubular organs may release ATP from epithelial cells

that then activates sensory nerve terminals. For example, epithelial cells in the bladder release ATP on distension, and the emptying reflex can be blocked by P2 receptor antagonists or when the P2X₃ receptor is knocked out (reviewed in Burnstock 2001; Deuchars 2001; Vlaskovska and others 2001). The actions of released ATP are thought to be terminated by extracellular ectonucleotidases that break ATP down.

Prior to this, investigations into ATP as a sensory mediator in the intestine began with Kirkup and colleagues (1999) who studied the extrinsic sensory neurons from the vagus (for review, see Grundy and Scratcherd 1997). The extrinsic sensory nerve terminals are activated by histamine (Kreis and others 1998) and, following the lead of Wood and colleagues who studied inflammation and cutaneous pain (reviewed in Wood and Docherty 1997), Kreis and colleagues (1998) believed that ATP might be co-released with histamine from mast cells. The activation of mast cells is known to enhance intestinal motility and secretion (Cooke 1994), thus making them prime candidates for the release of sensory mediators. At about the same time, believing ATP might be co-stored in some enteroendocrine cells with other sensory mediators (but not with 5-HT as EC cells do not contain ATP; Tamir and Gershon 1990), we began testing for a role of ATP as a sensory mediator at the terminals of the intrinsic sensory neurons (Bertrand and others 1999).

ATP Activates Intrinsic Sensory Nerve Terminals

In guinea pig ileum, application of ATP (1–2 mM in a pressure pipette) to regions of mucosa located circumferentially elicited trains of APs that were recorded at the myenteric sensory neuron cell body. These APs originated at the sensory terminals and were conducted to the cell body, where they were recorded with an intracellular electrode. The area of mucosa that ATP evoked APs from was usually only a few square millimeters. When electrical stimuli were applied to these same regions, an

antidromic AP occurred with a 3 to 7 ms latency; this suggests that the site of initiation is several millimeters away from the sensory neuron soma, which is consistent with the position of the stimulating electrode. These regions of mucosa were well correlated with areas from which 5-HT application or electrical stimulation evoked trains of APs (Bertrand and others 1999). This suggests that when ATP is applied to the mucosa, it interacts with specialized regions of mucosa that contain the terminals of sensory neurons. An analog of ATP, ATP- γ -S, produced a similar response, but other analogs, α,β -methylene-ATP and 2-methylthio-ATP, were only weakly active.

When compared to responses evoked by transient application of 5-HT or physiological stimulants to the mucosa, the ATP response was intermediate in the number of APs evoked, being less than that evoked by 5-HT but more than that evoked by physiological stimulants, acid, or acetate. The P2 receptor antagonist PPADS (60 μ M in the bath) abolished the APs evoked by ATP and ATP- γ -S (Fig. 5A); however, another P2 receptor antagonist suramin (100 μ M in the bath) had no effect. Either ATP or α,β -methylene ATP desensitized the ATP evoked response with a 50% recovery occurring after approximately 5 sec.

Taken together, the pharmacological properties, like those of the cell-body P2X receptors, do not match with known pharmacology. Given that the cell body of the sensory neurons expresses P2X₇ receptors, it is likely that P2X₇ receptors also exist at the sensory nerve terminal. Thus, some of the odd pharmacology might be explained if several P2X receptor subtypes, including the P2X₇ receptor, coexisted at the terminal.

Physiologically Released ATP and Enteric Reflexes

With the identification of ATP as a possible sensory mediator in the intestine, it is important to determine which reflexes are mediated by it. It is known that ATP evokes increases in internal calcium in mouse epithelial cells in small intestinal crypts (Sato and others 1999). Similarly, ATP in rat distal colon causes increases in internal calcium in crypt epithelial cells and secretion (Leipziger and others 1997). Kerstan and others (1998) have shown that endogenous ATP released from rat distal colon epithelium causes potassium secretion. Finally, Yu and others (2002) have found that chloride secretion and contraction of the smooth muscle is dependent on endogenously released ATP. In preliminary experiments, we found that blockade of P2X receptors in the lumen of the guinea pig ileum with PPADS had little effect on the pressure threshold for initiation of peristalsis. On the other hand, new data from Bian and others (2002), in mouse ileum, has shown that PPADS dramatically reduces the peak pressure response and the number of contractions during a 30 sec peristaltic reflex. Importantly, this effect of PPADS was only seen in control mice with an intact P2X₃ receptor (i.e., wild type, P2X₃^{+/+}) but not P2X₃^{-/-} knockout mice.

Interaction between ATP and EC Cells

5-HT and ATP have been identified as possible sensory mediators in the intestine. The question then arises as to whether they can both act directly at the nerve terminal or if one is simply releasing the other.

5-HT Does Not Release ATP

In the guinea pig intestine, we found no evidence that 5-HT stimulated the release of endogenous ATP. When PPADS (60 μ M in the bath) was present, ATP applied to the mucosa evoked trains of APs that were abolished, but there was no change in the number of 5-HT-evoked APs reaching the sensory neuron cell body (Fig. 5). This suggests that either 5-HT does not cause release of ATP at a site near the sensory nerve terminals or that any ATP released is surplus to the already maximal response evoked by 5-HT. In either case, these data are supportive of 5-HT acting directly on the nerve terminal. Recent data also support this view, as it has been shown that 5-HT₃ receptors are on the sensory nerve terminals and some enteroendocrine cells but not on EC cells (Mazzia and others 2003; Raybould and others 2003). Thus, 5-HT acts directly at the nerve terminal and does not appear to release any physiologically relevant ATP.

ATP Releases 5-HT

On the other hand, there is evidence to suggest that ATP can release 5-HT from EC cells. For instance, Xue and others (2002), studying the human carcinoid BON cell, which is a model for EC cell transduction events, have presented data that ATP can cause release of 5-HT. Also, data presented by Schwörer and others (1993) demonstrated that ATP can evoke release of 5-HT from pig EC cells. In support of this, we have found evidence that ATP causes the release of endogenous 5-HT. The selective 5-HT₃ receptor antagonist granisetron (1 μ M in the bath) consistently reduced the number of APs in a train evoked by ATP in a reversible manner, whereas the responses to 5-HT were blocked (Fig. 6). These data, combined with results from our previous study (Bertrand and others 2000), suggest that the actions of endogenously released 5-HT should also be blocked by granisetron and, thus, that the granisetron-resistant response to ATP is not mediated by 5-HT. Thus, 5-HT, released by ATP, activates the same sensory nerve terminals as ATP itself, suggesting that 5-HT₃ receptors and P2X receptors are colocalized on the same sensory nerve terminals (see Fig. 7).

Summary

In the ENS, ATP plays a role as a neurotransmitter between enteric neurons and as a sensory mediator from epithelial sources to the sensory nerve terminals. The regulated release of ATP from epithelial sources may play a role in the normal activation of intrinsic sensory nerve terminals in the ENS, thus participating in the

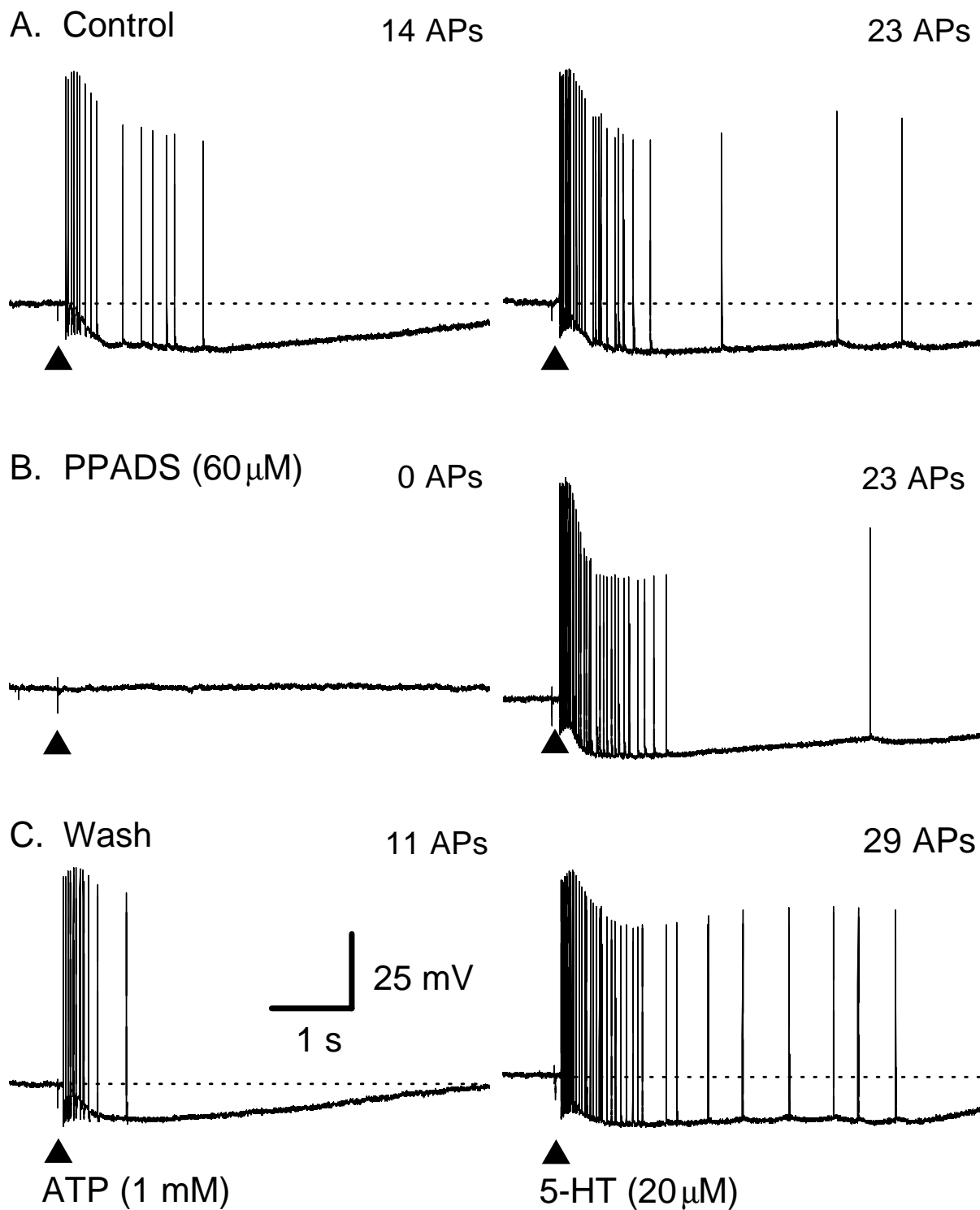
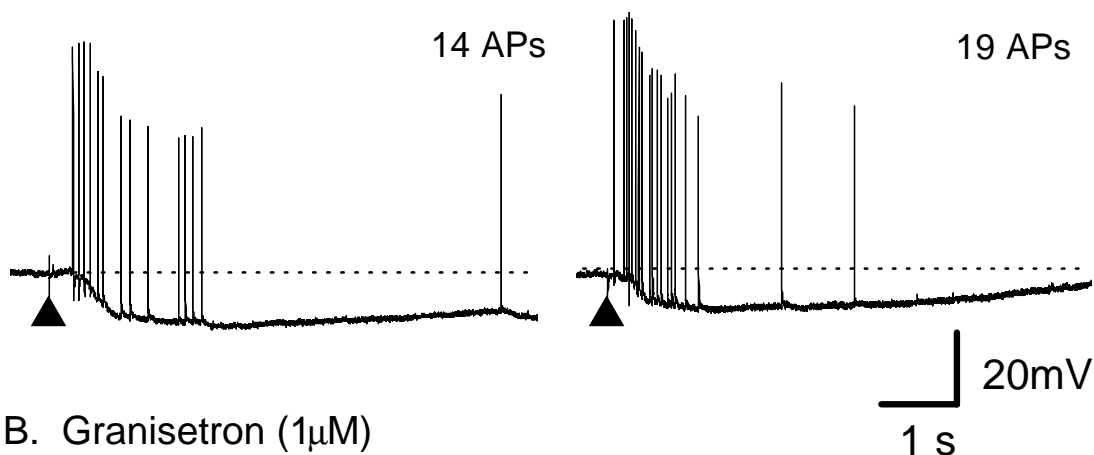
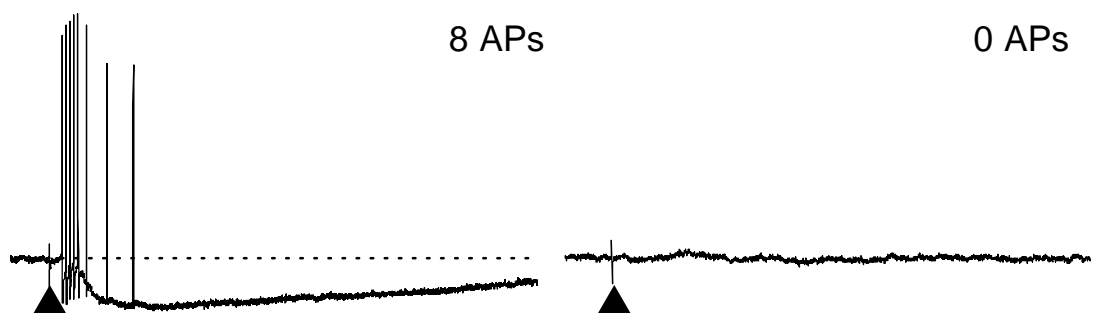


Fig. 5. The P2 receptor antagonist, pyridoxal phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), blocks the train of action potentials (APs) evoked by ATP but not by 5-hydroxytryptamine (5-HT). Representative voltage traces from a single intrinsic sensory neuron; dotted lines indicate resting membrane potential (RMP). The scale bars in C apply to all traces. The total number of APs is shown to the right of each trace. *Left. A*, ATP (2 mM, 100 ms pressure application to the mucosa, applied at the filled triangle) elicited a 1.8 sec duration train of 14 APs. *B*, The response was blocked by PPADS (60 μM). *C*, 25 minutes later following washout of PPADS, there is partial recovery of the response—a short train of APs has appeared (RMP: control = -63 mV, PPADS = -46 mV, recovery = -59 mV). *Right. A*, 5-HT (20 μM, 100 ms pressure application to the mucosa, applied at the filled triangle) elicited a train of 23 APs. *B*, The response was not affected during superfusion with PPADS (23 APs) and remained stable during washout of PPADS (*C*, 29 APs) (RMP: control = -64 mV, PPADS = -48 mV, wash = -56 mV). Adapted from Bertrand and Bornstein (2002), copyright by Society for Neuroscience.

A. Control



B. Granisetron (1 μM)



C. Wash

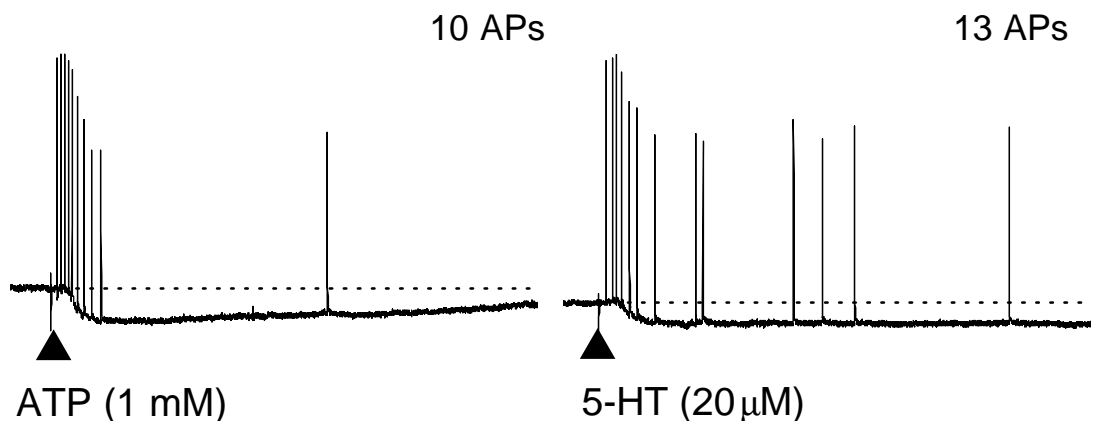


Fig. 6. The 5-HT₃ receptor antagonist, granisetron, blocks the trains of action potential (AP)-evoked 5-hydroxytryptamine (5-HT) and reduces those evoked by ATP. Representative voltage traces from a single intrinsic sensory neuron; the dotted lines indicate resting membrane potential (RMP). The scale bars in *A* apply to all traces. The total number of APs is shown to the right of each trace. *Left. A*, ATP (1 mM, 100 ms pressure application to the mucosa, applied at the filled triangle) elicited a train of 10 APs for a duration of 2.1 sec. Granisetron (1 μM) caused a significant reduction in the number of APs (8 APs, duration of 0.6 sec) (RMP = -77 mV). *C*, The train of APs recovered after washout of granisetron (9 APs, duration of 0.8 sec). *Right. A*, 5-HT (20 μM, 100 ms, applied at the filled triangle) elicited a train (15 APs, duration of 3.9 sec). *B*, Granisetron (1 μM) fully blocked this response. *C*, Twenty-five minutes after washout, the train of APs had partially recovered (8 APs, duration of 3.1 sec). Adapted from Bertrand and Bornstein (2002), copyright by Society for Neuroscience.

transduction of sensory stimuli and the initiation of enteric reflexes. ATP can act both directly through

P2X receptors on the sensory nerve terminal and through the release of serotonin from EC cells. Together,

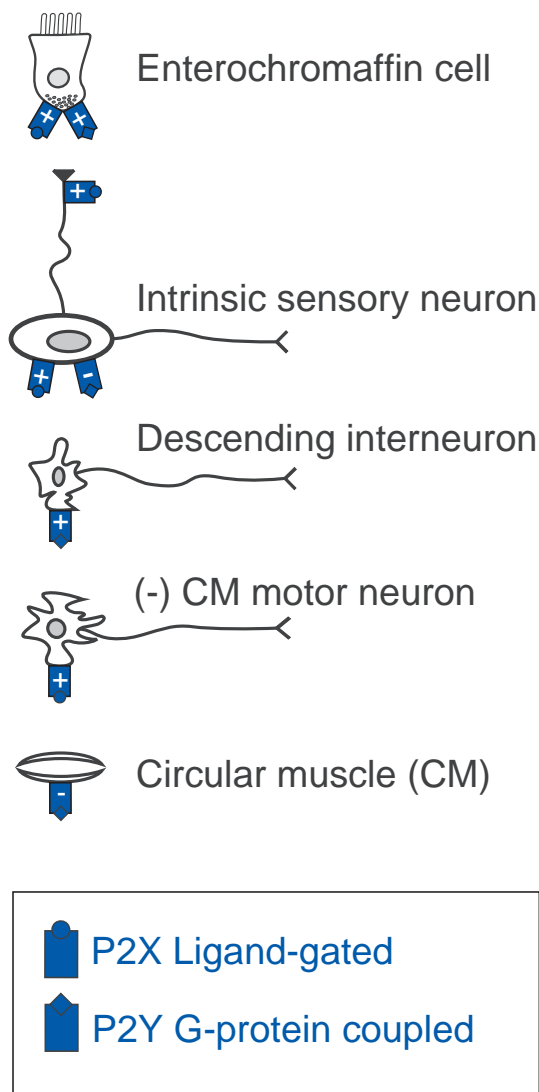


Fig. 7. Location of P2X receptors in the enteric nervous system. An illustration of functional classes of enteric cells to which the P2X (with a circle) and P2Y (with a square) receptors have been localized in the guinea pig ileum. A (+) indicates an excitatory receptor (coupled to a depolarization) and a (-) indicates an inhibitory receptor (one which couples to a hyperpolarization). From the top, the enterochromaffin cells have been found to have excitatory P2X and P2Y receptors. The intrinsic sensory neurons from the myenteric plexus have excitatory P2X receptors on the nerve terminals in the mucosa and on the cell body; also on the cell body are inhibitory P2Y receptors. Descending interneurons that stain positive for nitric oxide synthase have excitatory P2Y receptors while inhibitory motor neurons to the circular muscle, which also stain positively for nitric oxide synthase, have excitatory P2X receptors. Finally, circular smooth muscle has inhibitory P2Y receptors that mediate an active relaxation of the muscle.

there is a strong case that ATP participates in both the sensory transduction of stimuli from the gut lumen and in the subsequent initiation and propagation of enteric reflexes. For a focus on the circuitry in the submucosal plexus, the interested reader is directed to a recent

review by Cooke and colleagues (2003) that also explores the role of ATP in mechosensory transduction.

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