Synaptic Transmission at Functionally Identified Synapses in the Enteric Nervous System: Roles for Both Ionotropic and Metabotropic Receptors

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Abstract: Digestion and absorption of nutrients and the secretion and reabsorption of fluid in the gastrointestinal tract are regulated by neurons of the enteric nervous system (ENS), the extensive peripheral nerve network contained within the intestinal wall. The ENS is an important physiological model for the study of neural networks since it is both complex and accessible. At least 20 different neurochemically and functionally distinct classes of enteric neurons have been identified in the guinea pig ileum. These neurons express a wide range of ionotropic and metabotropic receptors. Synaptic potentials mediated by ionotropic receptors such as the nicotinic acetylcholine receptor, P2X purinoceptors and 5-HT₃ receptors are seen in many enteric neurons. However, prominent synaptic potentials mediated by metabotropic receptors, like the P2Y₁ receptor and the NK₁ receptor, are also seen in these neurons. Studies of synaptic transmission between the different neuron classes within the enteric neural pathways have shown that both ionotropic and metabotropic synaptic potentials play major roles at distinct synapses within simple reflex pathways. However, there are still functional synapses at which no known transmitter or receptor has been identified. This review describes the identified roles for both ionotropic and metabotropic neurotransmission at functionally defined synapses within the guinea pig ileum ENS. It is concluded that metabotropic synaptic potentials act as primary transmitters at some synapses. It is suggested identification of the interactions between different synaptic potentials in the production of complex behaviours will require the use of well validated computer models of the enteric neural circuitry.

Key Words: Ionotropic receptors, metabotropic receptors, enteric nervous system, intestinal reflexes, synaptic transmission, slow EPSPs, IPSPs, fast EPSPs.

INTRODUCTION

The gastrointestinal tract is essential for sustaining life. Its primary functions are the digestion and absorption of nutrients and the secretion and reabsorption of fluid. These depend on the contractile activity (motility) of the intestinal smooth muscle to mix and propel the intestinal contents in patterns appropriate to the physiological conditions. Both motility and fluid transport are regulated primarily by the myenteric and submucosal plexuses of the enteric nervous system (ENS) contained within the gastrointestinal wall and occur almost entirely without conscious control. In normal circumstances, intestinal function is probably regulated by a combination of intrinsic and extrinsic influences. However, a unique characteristic of the ENS is that it can regulate normal intestinal functions in the absence of any connection with external neural pathways.

The basic structure and some properties of the ENS have been extensively reviewed [31]. Much is known about the properties of individual enteric neurons, but how they fit into functional circuits and the nature of synaptic transmission at specific synapses during physiologically relevant behaviours remain areas of uncertainty. This has wide general interest, because many neurotransmitters important in the central nervous system are also found in enteric neurons and their physiological roles are often much easier to study in this peripheral neural network. Importantly, transmission within

the ENS clearly involves synaptic potentials mediated by metabotropic receptors as well as the conventional synaptic potentials mediated by ionotropic receptors. The former are difficult to study in the central nervous system, so there can be important lessons from an analysis of functional transmission within the ENS. This is the subject of this review.

STRUCTURE OF THE ENTERIC NERVOUS SYSTEM

The neuron cell bodies of the ENS are contained in two distinct ganglionated layers: the myenteric plexus between the longitudinal muscle and circular muscle layers, and the submucosal plexus between the circular muscle and the mucosa [31]. The myenteric plexus is largely responsible for regulation of intestinal motility, while the submucosal plexus is critical for the regulation of water and electrolyte secretion. However, there are extensive interconnections between the two layers with the myenteric plexus playing a key role in the coordination of motility and secretion.

TYPES OF ENTERIC NEURON

Many intestinal behaviours can be seen *in vitro*, i.e. in the absence of any neural input from outside the gastrointestinal tract, although these responses depend on the activity of the enteric neural circuitry. Such physiological studies lead logically to the idea that there are many different classes of enteric neurons falling into distinct functional groups (for reviews see [9,12,16,17,31]). These include one or more populations of intrinsic sensory (or primary afferent) neurons that respond to changes in muscle length or tension, to mucosal deformation and/or to chemical stimuli such as fatty acids and amino acids within the intestinal lumen. There are orally directed (ascending) and anally directed (descending) in-

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terneurons, excitatory motor neurons supplying the circular muscle and a separate population of excitatory motor neurons supplying the longitudinal muscle and populations of inhibitory motor neurons supplying each of these muscle coats. Secretion of water and electrolytes is regulated by at least two populations of secretomotor neurons, one cholinergic and the other non-cholinergic. Blood flow is partially regulated by enteric vasodilator neurons and some enteric neurons project out of the gut to make connections within prevertebral sympathetic ganglia, the intestinofugal neurons. Thus, at least 11 subtypes of enteric neurons can be deduced to be within the intestinal wall and the actual number is substantially larger (for reviews see [16,31]).

Extensive studies of the guinea-pig ileum have identified nearly all these different classes of enteric neurons by their characteristic neurochemistries or chemical codes (for detailed reviews see [16,17,32]). Such studies indicate that there are at least 20 neurochemically and functionally distinct classes of enteric neurons in this part of the guinea-pig gastrointestinal tract (see Table 1 of [16]). These studies, combined with electrophysiological studies of individual neurons during behavioural reflexes and immunohistochemical analyses of the locations of specific neurotransmitter receptor proteins (Table 1), provide a powerful tool for understanding the enteric neural circuitry and its dynamic properties. Unfortunately, the details of the neurochemical codes for apparently analogous neurons differ between intestinal regions [78] and between species [36], so this review will concentrate on the most comprehensively studied preparation, the guinea-pig ileum.

Table 1. Receptors Shown Immunohistochemically to be Expressed in Different Functional Subtypes of Enteric Neurons in Guinea-Pig Ileum

Neuron Type, Marker	Receptor Subtype	Reference
Myenteric AH/Dogiel type II, calbindin	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	GluR1	[69]
	GluR4	[69]
	NR1	[69]
	P2X ₂	[20]
	NK ₁	[73]
	NK ₃	[53]
	5-HT _{1A}	[64]
	5-HT ₄	[100]
	5-HT ₇	[128]
	P2Y ₁₂	[137]
	CRF1	[71]
	AT1	[131]
	BK2	[52]

(Table 1. Contd....)

Circular muscle inhibitory motor neuron, NOS	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	GluR2/3	[69]
	P2X ₂	[20]
	NK ₁	[6,73,102]
	NK ₃	[53]
	BK2	[52]
Excitatory motor neuron, circular muscle, ChAT/SP	AT1	[131]
	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	NK ₁	[73]
	CRF1	[71]
Excitatory motor neuron, longitudinal muscle, calretinin/ChAT	AT1	[131]
	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	P2X ₃	[99,129]
	GluR2/3	[69]
	GluR4	[69]
	NK ₃	[53]
	5-HT ₄	[100]
	AT1	[131]
	P2Y ₂	[136]
	P2Y ₆	[137]
Ascending interneuron, calretinin/ChAT	AT1	[131]
	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	P2X ₃	[99]
	GluR1	[69]
	GluR2/3	[69]
	NK ₃	[53]
Descending interneuron, NOS/VIP	AT1	[131]
	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	P2X ₃	[129]
	NK ₃	[53]
	P2Y ₆	[137]
	B2	[52]
Descending interneuron, somatostatin/ChAT	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	GluR1	[69]
Submucosal AH/Dogiel type II neuron, calbindin/substance P/ChAT/NeuN	GluR1	[69]

(Table 1. Contd....)

	GluR2/3	[69]
	GluR4	[69]
	NK ₁	[73,102]
	5-HT ₄	[100]
	AT1	[131]
	CRF1	[73]
Non-cholinergic secretomotor neuron submucosa, VIP	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	P2X ₂	[20]
	GluR2/3	[69]
	mGluR5	[69]
	CB1	[75]
Cholinergic secretomotor neuron submucosa, NPY	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	P2X ₃	[99]
	NK ₁	[73,86,102]
	P2Y ₂	[136]
	CRF1	[71]
	CB1	[75]
Cholinergic vasodilator neuron submucosa, calretinin	nAChR: $\alpha 3$, $\alpha 5$, $\beta 4$	[63]
	P2X ₃	[129]
	NK ₃	[53]

The table lists immunohistochemically identified receptors located in functional classes of neurons identified immunohistochemically in the guinea-pig ileum.

While neurochemistry, pharmacology (see below) and functional studies have all revealed a rich array of different classes of enteric neurons, electrophysiological studies have really only been able to divide these neurons into two distinct populations (see [11] for review), (Fig. 1). Neurons in one class are characterised by prolonged after-hyperpolarizing potentials (AHPs) that follow their action potentials, (Fig. 1C) [47] and have a common morphology being large oval shaped cells with several axons, (Fig. 1A) [14]. These neurons are found in both the myenteric plexus and the submucosal plexus [10] and are called AH/Dogiel type II neurons in the discussion below. The neurons in the other broad class typically lack prolonged AHPs following their action potentials, but exhibit prominent fast excitatory synaptic potentials, (Fig. 1B,C) [47]. These neurons nearly always have only a single axon, (Fig. 1A) [11], and are termed S/uniaxonal neurons in the discussion below. One major role of the AH/Dogiel type II neurons is as intrinsic sensory (or primary afferent) neurons (for reviews see [12,34,35], while S/uniaxonal neurons are interneurons, muscle motor neurons and secretomotor neurons [12,13,113]. However, while this division is convenient, it remains somewhat controversial because AH/Dogiel type II neurons receive excitatory synaptic

Fig. (1). The classification of enteric neurons according to their morphological and electrophysiological properties. A is a micrograph showing two different morphological types of myenteric neuron. The neurons were injected with biocytin during electrophysiological recordings and visualised using a fluorescence microscope after histological processing. Dogiel type I neurons are uniaxonal and have several short dendrites. Dogiel type II neurons have smooth cell bodies and multiple axons. Enteric neurons are classified as AH or S neurons according to their electrophysiological properties. The left panel in B shows a voltage recording of the response of an S neuron to a single electrical pulse, which evoked a fast EPSP (black arrow in B, left panel) that triggered an action potential (AP). S neurons do not show prolonged after-hyperpolarising potentials following APs (left panel in C). AH neurons rarely receive fast EPSPs (B right panel) and exhibit prolonged after-hyperpolarising potentials following APs (C right panel). Conveniently, S neurons have Dogiel Type I morphology and AH neurons display Dogiel type II morphology.

input (see below) and thus may act as interneurons under some conditions [123,124,133].

TYPES OF SYNAPTIC POTENTIALS

Four distinct classes of synaptic potentials have been identified in enteric neurons on the basis of their time courses and the directions of the underlying changes in membrane potential, (Figs. 2,3). There are three broad classes of excitatory synaptic potentials (EPSPs): fast EPSPs lasting 30 – 50 ms, (Fig. 2A), slow EPSPs lasting 5-10 s to

Fig. (2). Types of excitatory postsynaptic potentials in enteric neurons. Intracellular recordings showing a fast excitatory post synaptic potential (EPSP) (A) an intermediate EPSP (B) and a slow EPSP (C) recorded in two myenteric S/uniaxonal neurons and a myenteric AH/Dogiel type II neuron, respectively. Note the differences in time calibrations between the records.

minutes (Fig. 2C), and intermediate EPSPs that last 150 ms – 2.5 s (Fig. 2B). These can also be distinguished by their pharmacology and underlying conductance changes (see below). Inhibitory synaptic potentials (IPSPs), (Fig. 3), are also seen and can be subdivided on the basis of their pharmacology and sources.

In addition to the post-synaptic responses identified in enteric neurons, presynaptic inhibition mediated by muscarinic receptors [88], 5-HT_{1A} receptors [39,96], galanin [72], adenosine receptors (A1) [24] and α -adrenoceptors [39] has been described. Furthermore, facilitation of transmitter release *via* 5-HT₄ receptors appears to be a major mechanism within the ENS [41,96,97,127] and facilitatory presynaptic nicotinic receptors have been identified [108]. While these events are of considerable interest, their roles in specific pathways have not been identified and they will not be discussed further in this review.

Fast EPSPs and the Receptors that Mediate them

Fast EPSPs can be recorded in about 70% of all myenteric neurons and 90% of all submucosal neurons. They are

Fig. (3). Inhibitory postsynaptic potentials (IPSPs) in submucosal VIP neurons are reduced, but not abolished, by idazoxan. The trace in A shows an electrophysiological recording of an IPSP (arrow) evoked in a submucosal S neuron (later shown to be immunoreactive for VIP) by a train of 3 pulses to an internodal strand. B shows the same stimulus applied in the presence of the α_2 -adrenoceptor antagonist idazoxan (2 μ M), which reduces the IPSP indicating that it is partially mediated by noradrenaline released from extrinsic sympathetic nerves.

due to the opening of ligand-gated non-specific cation channels [38,40]. All myenteric S/uniaxonal neurons have fast EPSPs resulting from acetylcholine (ACh) acting on nicotinic acetylcholine receptors (nAChR), but fast EPSPs mediated by ATP acting at P2X receptors and by serotonin (5-HT) acting at 5-HT₃ receptors are also seen in myenteric S-neurons (for reviews [38,40]). Until recently, it was thought that fast EPSPs seen in submucosal S/uniaxonal neurons were exclusively due to activation of nAChR [85], but it has now been shown that P2X and 5-HT₃ receptors also play a role in some responses [82].

Immunohistochemical and molecular analyses have identified several different subunits of nAChR in the ENS (Table 1) [40,63]. It is generally agreed that the α_7 subunit is expressed by some enteric neurons, but it is unclear whether this receptor has a role in synaptic transmission. By contrast, evidence for a role of the α_3 and β_4 subunits in synaptic transmission is strong (for review see [40]). Other evidence indicates that β_2 subunits are expressed in enteric neurons [94] and the exact stoichiometry of enteric nAChR remains to be definitively identified.

Three different classes of P2X receptors have been identified in enteric neurons of the guinea-pig small intestine: P2X₂, P2X₃ and P2X₇ [20,50,99,129,136]. As indicated in Table 1, immunohistochemical studies have localised P2X₂ and P2X₃ receptors to different functionally defined subsets

of neurons in the guinea-pig ileum. Their roles will be discussed in more detail below.

Local application of 5-HT has shown that the ionotropic receptors for serotonin, 5-HT₃ receptors, are found on a large proportion of both myenteric and submucosal neurons [76,77,83,118,130,142]. However, neurons exhibiting EPSPs mediated by 5-HT₃ receptors are rare (< 10% of those studied) [142]. This illustrates a key problem in interpreting pharmacological data produced by application of agonists, or receptor localization data, in the ENS. It is easy to show that receptors are present, but it is much more difficult to show that they are ever exposed to significant concentrations of an endogenous ligand. Potential roles for EPSPs mediated by 5-HT₃ receptors will be discussed below.

It has been suggested that some myenteric and submucosal neurons have fast EPSPs mediated by ionotropic glutamate receptors [69]. However, despite convincing immunohistochemical evidence [69], electrophysiological analyses have not identified fast EPSPs mediated by glutamate in either myenteric or submucosal neurons [104]. This failure is puzzling, because there is pharmacological evidence that such receptors play a role in regulating intestinal behaviour [26,44].

Slow EPSPs, their Underlying Conductances and their Transmitters

Virtually every neuron within the ENS exhibits slow EPSPs as a result of electrical stimulation of interganglionic nerve trunks [11]. However, slow EPSPs evoked by physiological stimuli such as distension, mechanical stimulation of the mucosa or mucosal application of nutrients have been more difficult to identify. Time course and conductance measurements have identified three broad types of slow EPSPs. The first is typically evoked by a train of electrical stimuli applied to an interganglionic connective, lasts for 10s of seconds to minutes, (Fig. 2C) and is associated with a decrease in K⁺ conductance. These were first described in AH/Dogiel type II neurons by Wood and Mayer [135] and soon after in S/uniaxonal neurons [58,59]. A second type of slow EPSP can often be evoked by a single stimulus pulse applied to an interganglionic connective [7,15,51,82]. These are more rapid in onset and decay, lasting 10 – 30 s, and may be due to an increase in non-specific cation conductance [134]. This latter conclusion is controversial as some authors have reported slow EPSPs of this type associated with small decreases in membrane conductance, rather than the increases implied by this conclusion [7,93,117,125]. The third class of slow EPSP has been termed “sustained slow post-synaptic excitation” and is evoked by prolonged trains of relatively low frequency stimulation [2,25]. It is confined to AH/Dogiel type II neurons [1] and manifests as a very prolonged increase in neuronal excitability and an associated increase in input resistance.

Wood and Kirchgessner [134] reviewed the literature on the different classes of slow EPSP and concluded that they are associated with distinct intracellular transduction mechanisms. They suggest slow EPSPs involving K⁺ conductance decreases are mediated *via* adenylate cyclase and protein kinase A, while the shorter slow EPSPs associated with

cation conductance increases are mediated by phospholipase C and associated pathways. They also suggest that the two types are segregated according to electrophysiological class with AH/Dogiel type II neurons exhibiting slow EPSPs mediated by reductions in K⁺ conductance and S/uniaxonal neurons exhibiting slow EPSPs mediated by cation conductance increases. While this would be convenient, significant issues need to be resolved before it can be readily accepted. First, tachykinin receptors play a major role in the generation of slow EPSPs involving decreases in K⁺ conductance (see below) and are coupled to both adenylate cyclase and phospholipase C [89] with the latter being the preferred pathway in most instances [106,110]. In fact, some forms of the tachykinin receptors are exclusively coupled to phospholipase C [105]. Second, there have been many reports that slow EPSPs in S/uniaxonal myenteric neurons can involve reductions in K⁺ conductance or increased neuronal input resistance [15,43,59,93,125]. Third, many slow EPSPs in submucosal S/uniaxonal neurons are associated with increased input resistance and reverse at about the K⁺ equilibrium potential [7,117]. Indeed, it has been reported that both conductance increase and conductance decrease slow EPSPs are seen in the same submucosal neurons [51]. It seems likely that differences between slow EPSPs cannot be readily correlated with the broad electrophysiological subtypes of neurons. However, there is clear evidence for functional divisions of slow EPSPs according to physiological type (see below).

Nerve terminals within the ENS contain many different putative neurotransmitters, which mimic slow EPSPs when applied to enteric neurons (Table 2). In many cases, specific receptors for these putative transmitters have been identified immunohistochemically within neurochemically defined subtypes of enteric neurons (Table 1). However, every functionally defined subtype of enteric neuron expresses multiple types of receptor, including several metabotropic receptors. Thus, as many as 20 different transmitters may mediate slow EPSPs in one or more subpopulations of enteric neurons. Nevertheless, when electrically evoked slow EPSPs are studied using specific antagonists, they can be accounted for with only a restricted subset of the available putative transmitters. The roles of the other receptors and their associated endogenous ligands remain unclear.

Fig. (4) shows the bewildering variety of receptor subtypes identified pharmacologically or immunohistochemically in myenteric AH/Dogiel type II neurons of guinea-pig ileum. When slow EPSPs in these neurons are evoked by electrical stimulation of circumferentially directed pathways, virtually all are blocked by antagonists acting at NK₁ and NK₃ tachykinin receptors, (Figs. 5,6) [2,51]. There is also substantial evidence that 5-HT mediates slow EPSPs in these neurons, although the receptor subtype(s) responsible are less clearly defined. Earlier characterisation of slow EPSPs evoked by 5-HT implicated 5-HT_{1P} receptors [76,77,120], a receptor subtype identified pharmacologically, but which has not been cloned [49]. However, a more recent study concluded that stimulation of descending (anally directed) pathways evokes slow EPSPs in AH neurons *via* activation of 5-HT₇ receptors [83]. Unlike 5-HT_{1P} receptors, the 5-HT₇ receptor has been cloned (for review see [121]) and 5-HT₇

Table 2. Putative Mediators Found in Nerve Terminals within the ENS that are Known to Depolarize Enteric Neurons when Applied to their Cell Bodies

Mediator	Reference	Mediator	Reference
acetylcholine	[87]	ATP	[60]
Serotonin (5-HT)	[135]	Glutamate	[69]
γ -aminobutyric acid (GABA)	[21]	Histamine	[90]
noradrenaline	[107,119]	Adenosine	[22]
Angiotensin II	[131]	Bradykinin	[52]
Calcitonin gene-related peptide (CGRP)	[95]	Cholecystokinin	[109]
Corticotrophin releasing hormone	[70]	Gastrin releasing peptide	[141]
Neurokinin A	[79]	Pituitary adenylate cyclase activating peptide (PACAP)	[23]
somatostatin	[62]	Substance P	[61]
Thyrotrophin releasing hormone	[140]	Vasoactive intestinal peptide	[132,141]

receptor immunoreactivity in guinea pig myenteric AH neurons has been reported [128]. However, difficulties arise when interpreting the pharmacological data in relation to these receptor subtypes, because the commonly used agonist (5-CT) and antagonist (SB 269970) at 5-HT₇ receptors also have some affinity for 5-HT₁ receptors [37,46,49]. Furthermore, there is evidence that, in mouse myenteric neurons, a dimer of 5-HT_{1B/1D} receptors and the dopamine D₂ receptor mediates 5-HT-evoked slow EPSPs with similar properties to 5-HT_{1P} receptor mediated events [68]. Thus, the slow EPSPs mediated by these different receptor subtypes have similar characteristics and are difficult to distinguish pharmacologically. Consequently, it remains to be determined if more than one receptor subtype mediates 5-HT evoked slow EPSPs in guinea pig myenteric AH neurons or alternatively, if the 5-HT_{1P} receptor is actually a 5-HT₇ receptor or a heterodimer of 5-HT₁ receptors. The other transmitter known to mediate slow EPSPs in these neurons is acetylcholine acting on M₁ muscarinic receptors [91].

Slow EPSPs in S/uniaxonal neurons are more enigmatic. In the myenteric plexus, slow EPSPs mediated by NK₁ tachykinin receptors are seen in some S/uniaxonal neurons, which are immunoreactive for nitric oxide synthase (NOS) and are presumably inhibitory motor neurons [1,125]. Slow EPSPs mediated by P2Y₁ receptors are seen in about 50% of submucosal neurons, (Fig. 7), the non-cholinergic secretomotor neurons, in the guinea-pig ileum [51,82]. These neurons also exhibit slow EPSPs whose transmitter is unknown; because these neurons do not express NK₁, NK₂ or NK₃ tachykinin receptors [53,73,101,102], it is unlikely to be a tachykinin. We have unpublished data indicating that slow EPSPs mediated by P2Y₁ receptors can also be seen in some myenteric S/uniaxonal neurons, (Fig. 8), probably descending interneurons immunoreactive for NOS. Other data suggest that ascending interneurons and excitatory motor neurons exhibit slow EPSPs mediated by muscarinic receptors [57,93,126]. However, the full complement of mediators is

unclear and will remain so pending detailed analysis of the subtypes of S/uniaxonal neurons, an analysis hampered by the small size of many of these neurons.

Intermediate EPSPs

Electrical stimulation evokes EPSPs in some submucosal neurons that differ in both time course and underlying conductance change from either fast or slow EPSPs [7,82]. These intermediate EPSPs, as they are termed, last from 250 ms to 2 s, (Fig. 7), and are accompanied by a conductance increase, apparently for Cl⁻ ions. They are abolished by both the P2 receptor antagonist PPADS and the specific P2Y₁ receptor antagonist MRS 2179, and so are probably mediated by P2Y₁ receptors [82]. Our recent data indicates that P2Y₁ mediated intermediate EPSPs can also be seen in some myenteric S/uniaxonal neurons, (Fig. 8). Unlike myenteric neurons with P2Y₁ mediated slow EPSPs, myenteric neurons with intermediate EPSPs are not immunoreactive for NOS.

IPSPs and their Transmitters

Electrical stimulation evokes IPSPs in both myenteric and submucosal neurons, but the transmitters involved are distinctly different. The most prominent IPSPs in the ENS are seen in the VIP-immunoreactive secretomotor neurons of the submucosa [7,48]. These involve a substantial increase in membrane K⁺ conductance and are due the actions of two distinct neurotransmitters [8]. The most prominent component results from the activation of sympathetic nerve terminals and is due to noradrenaline acting at α_2 -adrenoceptors; it is profoundly depressed by the specific antagonist idazoxan, Fig. (3) [92]. There is, however, a second component of this response, which is thought to be due to somatostatin [112], although the receptor subtype has not been identified. The second component is mimicked by local application of either somatostatin or enkephalin, but opioid antagonists do not block the idazoxan resistant IPSP in these neurons, while somatostatin desensitization does [112].

Fig. (4). Identified receptors of myenteric AH / Dogiel type II neurons and their outputs to other enteric neurons. Receptor subtypes illustrated are those that have been identified either by neuropharmacology using specific agonists or antagonists, or by immunohistochemistry. They do not include receptors for compounds like vasoactive intestinal peptide that are known to depolarize these neurons through an unidentified receptor subtype. Identified receptors include several subclasses of serotonin receptor (5-HT_{1A}, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₇), cholecystokinin A and B receptors (CCK A and CCK B), receptors for ATP (P2X₂, P2X₇), N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors for glutamate, nicotinic (nAChR) and muscarinic (Musc) acetylcholine (ACh) receptors, neurokinin (NK)₁ and NK₃ tachykinin receptors, gamma-aminobutyric acid (GABA)_A receptors, angiotensin (AT)₁ receptors, corticotropin releasing factor (CRF)₁ receptors and bradykinin (BK)₂ receptors (see Table 1 for references). The AH/Dogiel type II neurons have identified outputs to ascending interneurons (Asc IN) via nAChR and muscarinic receptors, to other AH/Dogiel type II neurons via NK₁ and NK₃ tachykinin receptors (and possibly via muscarinic receptors), to descending interneurons (Desc IN) via an unknown transmitter/receptor combination (?) and to inhibitory motor neurons *via* an unknown transmitter/receptor combination.

IPSPs are more difficult to identify in the myenteric plexus. Nevertheless, they have been consistently reported in an ill-defined subset of myenteric neurons [24,54,59]. Both AH/Dogiel type II and S/uniaxonal neurons exhibit IPSPs, but there has been no systematic study of their incidence in the large number of neurochemically defined subtypes of the latter. Many AH/Dogiel type II neurons exhibit IPSPs, especially when slow EPSPs are blocked by tachykinin receptor

Fig. (5). Slow EPSPs in AH/Dogiel type II neurons evoked by electrical stimulation of circumferential internodal strands can be mediated by tachykinins acting at NK₃ receptors. A shows a slow EPSP evoked in an AH/Dogiel type II neuron by a train of 10 pulses (20 Hz) delivered to a circumferentially directed internodal strand. B shows the same stimulus delivered in the presence of the tachykinin NK₃ receptor antagonist SR 142801 (100 nM), which abolished the slow EPSP.

antagonists [54] or depressed by the presynaptic inhibitory effect of adenosine acting on A₁ receptors [24]. Unlike the IPSPs in the submucous plexus, IPSPs in myenteric AH/Dogiel type II neurons are resistant to blockade of α -adrenoceptors [54]. To date, the only data identifying a possible transmitter mediating the myenteric IPSPs comes from a limited study, which found that the 5-HT_{1A} receptor antagonist NAN-190 blocked IPSPs in some AH/Dogiel type II neurons [54]. Many AH/Dogiel type II neurons express 5-HT_{1A} receptors [64] and are hyperpolarized by 5-HT acting on these receptors [39,42]. Electrical stimulation of descending pathways including the axons of neurons containing 5-HT evokes slow EPSPs in many AH/Dogiel type II neurons [83] and blockade of 5-HT₇ receptors can convert the slow EPSPs into slow hyperpolarizations. Together these data suggest that some IPSPs in myenteric neurons are mediated

Fig. (6). Slow EPSPs in myenteric AH/Dogiel type II neurons evoked by electrical stimulation of the mucosa are largely mediated via NK_1 receptors. The top diagram shows a preparation in which the mucosa and submucosa are removed on one half of a segment of intestine to allow access to the myenteric plexus. The mucosa is intact on the circumferentially adjacent half. Myenteric neurons are impaled close to the intact mucosa and focal electrical stimuli applied to the mucosa near the impaled neurons. **A.** A slow EPSP evoked in an AH/Dogiel type II neuron by 10 electrical pulses (20 Hz) delivered to the mucosa. Action potentials evoked by the stimulus were followed by a large after-hyperpolarization before the slow EPSP was observed. Hyperpolarizing current pulses were delivered through the recording electrode during the response to analyse the underlying conductance changes. An AP can also be seen during the falling phase of the slow EPSP in this trace. **B.** The slow EPSP was unchanged in the presence of the NK_3 receptor antagonist SR 142801 (100 nM). **C.** The slow EPSP was abolished by the NK_1 receptor antagonist SR 140333 (100 nM).

by 5-HT acting on 5-HT_{1A} receptors, but no systematic analysis of all myenteric IPSPs has been undertaken.

IDENTIFYING THE ROLES OF SPECIFIC CLASSES OF SYNAPTIC POTENTIALS AT FUNCTIONALLY DEFINED SYNAPSES

There have been many pharmacological studies aimed at identifying the roles of different neurotransmitters in intestinal behaviours. They typically involve bath or systemic ap-

Fig. (7). P2Y receptors mediate intermediate and slow EPSPs evoked by electrical stimulation in VIP secretomotor neurons. **A.** Electrophysiological recording from a submucosal VIP neuron in response to a single electrical pulse delivered to an internodal strand, which elicited a fast EPSP and action potential followed by an intermediate EPSP (see dotted box expanded trace, black arrow) and a slow EPSP (grey arrow). In **B.** the P2 receptor antagonist PPADS (30 μM) abolished both the intermediate EPSP (black arrow) and the slow EPSP (grey arrow) suggesting that both were P2Y receptor mediated events. In this example, the fast EPSP/AP complex was unaffected by PPADS suggesting the fast EPSP was not mediated by P2X receptors.

plication of an antagonist to modify a functional parameter such as muscle contractility, intestinal propulsion or secretion. However, this does not allow for the wide distribution of different receptor subtypes, (Fig. 4) (Table 1), the complexity of the enteric neural circuitry or the fact that the elements of these circuits are usually intermingled within the same enteric ganglia. Thus, such studies can test if one or more synapses involving a particular transmitter are critical for a specific function, but cannot determine where the critical synapse is within the relevant neural circuit. The problem can be overcome by using knowledge of relevant pathways to apply antagonists to specific points within functional circuits and this section focuses on studies that identified the roles of specific synaptic potentials within the ENS.

Circuits Regulating Motility Reflexes

It has been known for over a century that local mechanical or chemical stimulation of the intestinal wall evokes contraction oral to the stimulus (ascending excitation) and relaxation anal to the stimulus (descending inhibition) [3]. These two reflexes have been thought to underlie virtually all intestinal motor behaviour and the elements of the relevant neural circuitry have been identified in guinea-pig ileum (for detailed review see [12]). Both the ascending excitatory pathway and the descending inhibitory pathway are feed

Fig. (8). P2Y₁ receptors mediate intermediate EPSPs or slow EPSPs in some myenteric S/uniaxonal neurons. A and B show recordings from a myenteric S/uniaxonal neuron, in which an intermediate EPSP (black arrow) was elicited by a single electrical stimulus (thin arrow under recording) to the mucosa (see Fig. 6). The intermediate EPSP was abolished by the specific P2Y₁ receptor antagonist MRS 2179 (10 μ M) (B). In another S neuron, the same type of stimulus evoked a slow EPSP (black arrow in C) which was also abolished by MRS 2179 (D).

forward circuits in which intrinsic sensory neurons excite interneurons running orally (ascending, Fig. 9) or anally (descending, Fig. 10). The interneurons act to excite other interneurons of the pathway and motor neurons supplying the circular muscle, with ascending interneurons contacting excitatory motor neurons and descending interneurons contacting inhibitory motor neurons. In guinea-pig ileum, both ascending and descending interneurons excite the excitatory motor neurons innervating the longitudinal muscle [113]. Because the interneurons run for significant distances within the myenteric plexus before contacting the motor neurons, the output of the reflex pathways is physically separated from the neural elements involved in sensory transduction. Several studies have exploited this to specifically analyse transmission at each point within the ascending and descending reflex pathways. This has been accomplished by dividing the organ bath which contains an intestinal preparation, so that

stimuli are applied in one chamber and recordings are made in a separately perfused chamber, (Fig. 11A) [45,126, 139]. Frequently, a third chamber separates the recording and stimulation chambers, (Fig. 11A). Antagonists can be added separately to the site of stimulation, the site of recording or at a point where they can only affect transmission along the reflex pathway excited by the stimulus. In the simplest case, this allows the effects of antagonists on sensory transduction and transmission from intrinsic sensory neurons to interneurons to be studied in the stimulation chamber, transmission between interneurons to be studied in the central chamber and transmission to motor neurons to be studied in the recording chamber.

The results of the initial studies of these pathways have been reviewed elsewhere [12] and are only summarised here (Fig. 9). Transmission from intrinsic sensory neurons sensi-

Fig. (9). A schematic of the ascending excitatory reflex pathway and identities of neurotransmitters acting at specific synapses within it. Transmission between myenteric AH/Dogiel type II neurons and ascending interneurons (Asc IN) occurs via ACh acting at nicotinic (nAChRs) and/or muscarinic receptors and by tachykinins acting at NK₃ receptors. Transmission between ascending interneurons is mediated via nAChRs and between ascending interneurons and excitatory motor neurons (EMNs) is via nAChRs and NK₃ receptors.

Fig. (10). Schematic of the descending reflex pathways and the identities of neurotransmitters acting at specific synapses within them. The transmitter(s) acting at synapses between intrinsic sensory neurons (myenteric AH / sensory neuron) and 3 classes of descending interneuron (IN) - those immunoreactive (IR) for nitric oxide synthase (NOS), vasoactive intestinal peptide (VIP), and gastrin releasing peptide (GRP), those IR for choline acetyltransferase (ChAT) and serotonin (5-HT), and those IR for ChAT and somatostatin (SOM) (not illustrated) - or between sensory neurons and inhibitory motor neurons (IMN), remain to be identified. ATP acting at P2Y₁ receptors may mediate transmission between NOS-descending interneurons during inhibitory reflexes. Transmission between descending interneurons and inhibitory motor neurons is via ATP acting at P2X receptors. ATP acting at P2X receptors and 5-HT acting at 5-HT₃ receptors are important in the descending excitatory reflex pathway, but their locations are unknown. NOS-descending interneurons connect ascending interneurons via an unidentified transmitter.

tive to intestinal distension (probably myenteric AH/Dogiel type II neurons) to ascending interneurons appears to be mediated by ACh acting at nAChR, by ACh acting at muscarinic receptors and by a tachykinin acting at NK₃ receptors [55,126]. It is only abolished when all three receptor subtypes are blocked. Transmission between ascending interneurons and other ascending interneurons is *via* nAChR [55], while transmission between ascending interneurons and excitatory motor neurons supplying the circular muscle is *via* nAChR and NK₃ tachykinin receptors [126]. The pharmacology at specific synapses within the ascending reflex pathway excited by mucosal deformation is identical to that of the equivalent synapses of the pathway excited by distension [126], although the intrinsic sensory neurons involved are clearly distinct populations [12,35,113]. Thus, fast EPSPs mediated by nAChR play a role at every functionally defined synapse within the ascending excitatory pathway, but metabotropic receptors, and presumably slow EPSPs, are also important at some synapses. As yet, slow EPSPs evoked by either distension or mucosal deformation have not been definitively recorded in neurons identified as being in the ascending excitatory reflex pathway.

The descending inhibitory reflex pathway is significantly more complex for several reasons. First, there are several different classes of descending interneurons (Fig. 10) [16, 17], while there is only one class of ascending interneurons [16,17]. Second, while electrical stimulation evokes fast EPSPs mediated by nAChR in all S/uniaxonal neurons of the

descending inhibitory pathway, many of these neurons also exhibit fast EPSPs mediated by either P2X or 5-HT₃ receptors [56,67,142]. Third, some sensory neurons have long anally projecting axons as well as circumferentially projecting axons [18], which complicates interpretation of data arising from pharmacological studies designed to isolate specific classes of synapses.

Initial studies of the descending inhibitory pathways in the guinea-pig ileum excited by either distension or mucosal deformation demonstrated that unlike the ascending pathway nAChR play very little part in transmission within this pathway [55,57]. A later study showed that transmission from descending interneurons to inhibitory motor neurons was largely, if not exclusively, due to fast EPSPs mediated by P2X receptors [5]. Inhibitory motor neurons are immunoreactive for NOS [19] and the great majority of NOS neurons are immunoreactive for P2X₂ receptors [20]. Thus, purinergic transmission from interneurons to inhibitory motor neurons is probably *via* the P2X₂ subtype. Interestingly, this was the first demonstration anywhere in the nervous system that P2X receptors played a major physiological role in a well defined behaviour.

The same studies demonstrated that transmission from intrinsic sensory neurons to descending interneurons of the inhibitory reflex pathway is not due to nAChR or P2X receptors (Fig. 10) [5,56]. Although a component of transmission from the intrinsic sensory neurons responding to mucosal

Fig. (11). P2Y receptors are involved in transmission between descending interneurons in descending reflexes evoked by distension. **A.** Depicts a 3-chambered organ bath (chambers 15 mm wide) and the inhibitory neural pathways activated by distension in the far and near distension chambers. The different classes of neuron are shown on the **left**. Inhibitory junction potentials (IJPs) are recorded from the circular muscle in the anal chamber in response to distension (**right**). The “X” marks the location of synapses between descending interneurons. **B.** Control recordings (upper traces) of IJPs evoked by far distension (**left panel**) and near distension (**right panel**). PPADS (30 μ M) in the near distension chamber (lower traces) reduced the IJP evoked by far distension (**left panel**), but had no effect on the IJP evoked by near distension (**right panel**).

deformation involves NK₃ tachykinin receptors, a significant component of this transmission is unaffected by NK₃ receptor blockade [55]. No transmitter/receptor combination studied has been found to be involved in transmission from the distension-sensitive intrinsic sensory neurons to descending interneurons of the inhibitory pathway. Roles for ACh, ATP, 5-HT, glutamate and the tachykinins as transmitters at this synapse have been ruled out. It has been proposed that transmission at these synapses is due to the actions of calcitonin gene-related peptide (CGRP) [45]. However, immunohistochemical studies have not identified CGRP in intrinsic sensory neurons of guinea-pig ileum, although it is present in the equivalent neurons of the mouse enteric nervous system [36]. Further, we recently tested this idea in the guinea-pig ileum and were unable to block relevant reflexes with a standard CGRP antagonist (unpublished data).

Recent results have begun to shed light on transmission between descending interneurons of the inhibitory pathway (Fig. 10). In particular, distension in the oral chamber of a divided organ bath evokes a slow EPSP in some neurons in the anal chamber [125]. These neurons were identified by intracellular injection of a neural marker, biocytin, as having the morphology of interneurons and were immunoreactive for NOS, which labels a subpopulation of descending in-

terneurons [16]. The slow EPSPs are abolished if all synaptic transmission is blocked in the stimulation chamber indicating that they result from release of transmitter from interneurons, rather than from the activity of intrinsic sensory neurons that project into the recording chamber. These slow EPSPs are not blocked by antagonists acting at muscarinic receptors, at NK₁ or NK₃ tachykinin receptors or at class 1 metabotropic glutamate receptors. However, unpublished data indicates that they are blocked by PPADS and they are very similar to the slow EPSPs shown in (Fig. 8), which are abolished by blockade of P2Y₁ receptors. Furthermore, both blockade of P2 receptors with an equivalent concentration of PPADS and blockade of P2Y₁ receptors in the intermediate, interneuron, chamber of a divided organ bath markedly depresses descending inhibitory reflexes (Fig. 11). Thus, transmission from a population of descending interneurons to NOS-immunoreactive descending interneurons is essential to the descending inhibitory reflex pathway and occurs *via* a slow EPSP mediated by the metabotropic P2Y₁ receptor. There is no evidence for involvement of fast EPSPs in this process, which represents the first clear indication that metabotropic receptors can play a primary role in transmission on their own.

Monosynaptic Transmission from Intrinsic Sensory Neurons to Inhibitory Motor Neurons

As stated above, many intrinsic sensory neurons, those that respond to distension, have long anally projecting axons. These may have monosynaptic connections with inhibitory motor neurons [55]. The studies discussed above failed to identify the transmitter operating at these synapses, although they excluded ACh, ATP (at P2X or P2Y₁ receptors), 5-HT, a tachykinin (NK₁ or NK₃ receptors), glutamate (AMPA, NMDA or class 1 metabotropic glutamate receptors) [5,55, 139]. Thus, the nature of transmission from intrinsic sensory neurons to inhibitory motor neurons in the guinea-pig ileum remains a mystery.

Transmission between AH/Dogiel Type II Neurons and its Potential Functions

While the reflex pathways are simple feed forward circuits, AH/Dogiel type II neurons predominantly project circumferentially and contact other AH/Dogiel type II neurons as well as the ascending and descending reflex pathways (Fig. 4). Simultaneous recordings from two of these neurons that are connected morphologically showed that stimulating the presynaptic neuron evoked slow EPSPs in the postsynaptic neuron [66]. These slow EPSPs appear to be mediated by tachykinins acting *via* NK₁ receptors, NK₃ receptors or both [54]. Stimuli applied to the mucosa also evoke slow EPSPs in these neurons, but these are specifically mediated by NK₁ receptors and not by NK₃ receptors (Fig. 6). Furthermore, activation of long anally projecting pathways evokes slow EPSPs blocked by the 5-HT₇ selective antagonist SB 269970 in these neurons [83]. Thus, AH/Dogiel type II neurons receive slow EPSPs from three distinct pathways. The roles of the slow EPSPs from descending pathways and from pathways running from the mucosa are unknown, and they have only recently been identified. However, the roles of the slow EPSPs, and hence tachykinergic transmission, arising from other myenteric AH/Dogiel type II neurons have been investigated by the use of anatomically and synaptically realistic computer simulations. As mentioned earlier, AH/Dogiel type II neurons act as intrinsic sensory (or primary afferent) neurons under the right physiological conditions [34,35]. The simulations indicate that the slow EPSPs evoked in these neurons by activity in their neighbours are essential for encoding the magnitudes of ongoing stimuli as would be seen under physiological conditions [122,123]. Thus, slow EPSPs and the metabotropic receptors that mediate them are essential for the sensory functions of the AH/Dogiel type II neurons.

The simulation studies also indicate that, under a different set of conditions, slow EPSPs in AH/Dogiel type II neurons allow them to act as coordinating interneurons that carry activity slowly along the intestine in the absence of sensory input [124]. This may be the mechanism underlying the interdigestive migrating motor complex. If so, then this represents another key role for metabotropic transmission within the enteric neural circuitry.

The Descending Excitatory Reflex Pathway

Relatively recently, another reflex pathway that regulates motility in the guinea-pig small intestine was identified in

physiological experiments [81,114-116]. This is the descending excitatory reflex: both distension and mucosal deformation can excite contractions of the circular and longitudinal muscle layers anal to the site of stimulation. Unlike the ascending excitatory and descending inhibitory pathways, descending excitation may not be due to a simple feed forward circuit. While anatomical connections have been described between anally projecting neurons and neurons of the excitatory pathways, these are synapses between descending NOS interneurons and ascending interneurons [98]. Thus, descending excitation may be due to a recurrent loop with the pathway first running anally and then turning orally (Fig. 10).

Studies of transmission in the descending excitatory pathway have been limited, but enlightening. This reflex is absent if contractile activity is blocked [115], which has prevented the type of detailed studies that clarified the ascending excitatory and descending inhibitory pathways. Nevertheless, available data indicate a key role for purinergic transmission, perhaps *via* P2X receptors, between descending interneurons in the pathway and, importantly, also for 5-HT₃ receptor mediated transmission [81,116]. Blockade of P2 receptors with a concentration of PPADS that is relatively specific for P2X receptors depresses descending excitation of both the longitudinal and circular muscle. By contrast, blockade of 5-HT₃ receptors depresses reflex responses of the circular muscle, but has no effect on the longitudinal muscle. Reports of the effects of blockade of nAChR are inconsistent, which may reflect the different stimuli used to evoke the reflexes studied. Nevertheless, the major form of transmission along the pathway is clearly mediated by P2 receptors, but there is a key synapse at which fast EPSPs mediated by 5-HT₃ receptors are essential in the pathway exciting the circular muscle.

The finding that 5-HT₃ receptors are important in a descending reflex pathway suggests a role for a population of descending interneurons that has been known for 25 years [33] and been enigmatic for the same period. The only neurons within the ENS that contain 5-HT are descending interneurons that are also immunoreactive for choline acetyltransferase (ChAT) [16] and hence may be cholinergic. These neurons are likely to be responsible for the slow EPSPs resulting from activation of 5-HT₇ receptors in the AH/Dogiel type II neurons, but only make sparse contact with these neurons [29,138]. About 10% of S/uniaxonal neurons exhibit 5-HT₃ mediated fast EPSPs in addition to nicotinic fast EPSPs [142] and is it probable that these neurons play a critical role in descending excitation.

Secretomotor Pathways

Mucosal secretion of water and electrolyte is largely mediated by neurons with cell bodies in the submucosal plexus [31]. In guinea-pig ileum, there are 4 classes of neurons within the submucosa: AH/Dogiel type II neurons, cholinergic secretomotor neurons that are immunoreactive for neuropeptide Y, non-cholinergic secretomotor neurons immunoreactive for vasoactive intestinal peptide (VIP) and cholinergic vasodilator neurons immunoreactive for calcitonin (for details see [32]).

The non-cholinergic secretomotor neurons are the most interesting as they receive converging input from several

different sources. These neurons receive fast EPSPs mediated *via* nAChR from anally projecting myenteric neurons, as well as fast EPSPs from submucosal neurons (probably the AH/Dogiel type II neurons, which are immunoreactive for both tachykinins and choline acetyltransferase) [84,85, 103]. They also exhibit fast EPSPs mediated by P2X receptors, probably P2X₂ receptors as these are expressed by all submucosal VIP neurons [82]. These neurons also exhibit intermediate and slow EPSPs mediated by P2Y₁ receptors [51,82], but the sources of these inputs are unclear. Furthermore, the VIP neurons respond to trains of stimuli with slow EPSPs that are not purinergic and whose transmitter(s) have been difficult to identify. The VIP neurons are not immunoreactive for any of the tachykinin receptors [53,73,101, 102], so it is unlikely that the non-purinergic slow EPSPs are due to a tachykinin. However, some neurons responsive to NK₁ receptor specific agonists are not immunoreactive for NK₁ tachykinin receptors [54,86]. Non-purinergic slow EPSPs in VIP neurons are mimicked by several agonists including VIP itself [111] and it has been postulated that VIP arising from recurrent connections from other VIP neurons might mediate these slow EPSPs [65]. This remains to be definitively tested. Other candidates include class I metabotropic glutamate receptors, as slow EPSPs mediated by such receptors, probably the mGluR5 subtype, have been reported [104].

VIP secretomotor neurons also exhibit two pharmacologically distinct IPSPs (Fig. 3): one mediated *via* α_2 -adrenoceptors [92] and the other apparently by somatostatin [112]. They are the only submucosal neurons to receive direct synaptic connections from sympathetic neurons and thus are an important regulatory target for control of whole body water and electrolyte balance. This malfunctions during secretory diarrhoea due to bacterial toxins, which typically act by greatly increasing firing of VIP neurons [30,74]. Sympathetic nerve terminals supplying the submucosal plexus are immunoreactive for somatostatin [27], which raises the possibility that the two types of IPSP arise from the same nerve terminals. However, non-adrenergic IPSPs persist after extrinsic denervation [8,80], but are abolished when the overlying myenteric plexus is also removed to cause degeneration of all synaptic terminals arising from cell bodies outside the submucosa [8]. Thus, the non-adrenergic IPSP arises from myenteric neurons. Myenteric somatostatin neurons are known to project to the submucosa [28], but are also immunoreactive for ChAT [16] and may be cholinergic. This raises the possibility that inputs from somatostatin neurons may be both excitatory and inhibitory on the same VIP neuron.

Other submucosal neurons also have diverse inputs. For example, NPY cholinergic secretomotor neurons exhibit fast EPSPs [7] and these probably include responses mediated by both nAChR and 5-HT₃ receptors [82]. The latter arise from myenteric neurons, because the only 5-HT neurons in the ENS have their cell bodies in the myenteric plexus [16], but the source(s) of the former remain unclear. NPY neurons also exhibit slow EPSPs in response to trains of electrical stimuli [7], but the transmitters responsible are unknown. Immunohistochemical studies have identified NK₁ and NK₃ tachykinin receptors in these neurons [53,73], but they also

express P2Y₂ receptors [136], (Table 1). Whether any of these mediates physiologically evoked slow EPSPs remains to be determined.

Inputs to submucosal AH/Dogiel type II neurons are less well defined. They exhibit slow EPSPs in response to trains of stimuli [10] and express immunoreactivity for NK₁ [73], 5-HT₄ [100], GluR1 [69] and CRF1 receptors [71] (Table 1). Some excitatory input comes from myenteric AH/Dogiel type II neurons [42], which express tachykinins [16], suggesting that the slow EPSPs are mediated *via* NK₁ receptors. However, this remains to be established.

These observations underline the conclusion that both ionotropic receptors and metabotropic receptors play critical roles at synapses within the neural circuits that regulate gastrointestinal functions.

TRANSLATING SYNAPTIC TRANSMISSION TO INTESTINAL BEHAVIOUR

The studies that form the basis of this review have largely involved either electrical stimulation of nerve trunks within the enteric neural plexuses or physiological stimuli applied locally to excite simple stereotyped reflexes. However, normal intestinal behaviours are considerably more complex. For example, while ascending excitation, descending inhibition and descending excitation may underpin intestinal propulsion, the major motor activity of the small intestine after a meal is segmentation, which involves localised stationary contractions in response to a widespread stimulus. In the fasted state, the major motor pattern is the interdigestive migrating motor complex, characterised by bands of strong contractions that propagate very slowly along the intestine. Neither behaviour can be easily explained by the simple feed forward or open recurrent circuits responsible for local reflexes [124]. This raises the question as to how the specific synaptic potentials discussed in this review fit into the neural circuitry regulating the more complex behaviours of the intestine. However, there are few tools available as yet that allow this issue to be adequately addressed, because the more complex behaviours are only seen in preparations in which it is not possible to record from individual neurons.

A further problem is that there are few transmitter/receptor combinations unique to a single functional class of synapse within the enteric circuitry. For example, nAChR are found on virtually every class of enteric neurons (Table 1). Similarly, NK₁ tachykinin receptors are important for transmission between intrinsic sensory neurons [54] and for transmission to inhibitory motor neurons [1,125]. Further, many of these combinations are also implicated in neuromuscular transmission (e.g. ATP at P2 receptors [9]) or sensory transduction (5-HT at 5-HT₃ receptors [4]).

The solution may be to use well validated anatomically and synaptically realistic models to predict the consequences of modulation of activity at functionally identified synapses on the more complex behaviours. However, development of such models is still in its infancy and further work is required to both characterise specific forms of synaptic transmission and incorporate these into valid models of intestinal behaviour. This is essential to determine the roles of the

ionotropic and metabotropic synaptic potentials characteristic of the enteric neural circuitry and suggest how these may interact in more complex systems.

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