Electrical stimulation of the mucosa evokes slow EPSPs mediated by NK1 tachykinin receptors and by P2Y₁ purinoceptors in different myenteric neurons

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Gwynne RM, Bornstein JC. Electrical stimulation of the mucosa evokes slow EPSPs mediated by NK1 tachykinin receptors and by P2Y₁ purinoceptors in different myenteric neurons. Am J Physiol Gastrointest Liver Physiol 297: G179–G186, 2009. First published April 30, 2009; doi:10.1152/ajpgi.90700.2008.—Slow excitatory postsynaptic potentials (EPSPs) in enteric neurons arise from diverse sources, but which neurotransmitters mediate specific types of slow EPSPs is unclear. We investigated transmitters and receptors mediating slow EPSPs in myenteric neurons evoked by electrical stimulation of the mucosa in guinea pig small intestine. Segments of ileum or jejunum were dissected to allow access to the myenteric plexus adjacent to intact mucosa, in vitro. AH and S neurons were impaled with conventional intracellular electrodes. Trains of stimuli delivered to the mucosa evoked slow EPSPs in AH neurons that were blocked or depressed by the neurokinin-1 (NK1) tachykinin antagonist SR140333 (100 nM) in 10 of 11 neurons; the NK3 tachykinin receptor antagonist SR142801 (100 nM) had no effect on slow EPSPs in seven of nine AH neurons. Single pulses to the mucosa evoked fast EPSPs and slow depolarizations in S neurons. The depolarizations were divided into intermediate (durations 300–900 ms) or slow (durations 1.3–9 s) EPSPs. The slow EPSPs were blocked by pyridoxal phosphate-6-azophenyl-2-4-disulfonic acid (30 μM, N = 3) or the specific P2Y₁ antagonist MRS 2179 (10 μM, N = 6) and were predominantly in anally projecting S neurons that were immunoreactive for nitric oxide synthase (NOS). In contrast, intermediate EPSPs were predominantly evoked in NOS-negative neurons; these were abolished by MRS 2179 (N = 8). Thus activation of pathways running from the mucosa excites three different types of slow EPSPs in myenteric neurons, which are mediated by either a tachykinin (NK1, AH neurons) or a purine nucleotide (P2Y₁, S neurons).

AH neurons; S neurons; ATP; neurokinin-1

THE NEURAL PATHWAYS CONTROLLING intestinal motility are contained within the myenteric plexus of the enteric nervous system (ENS). These pathways are activated by various stimuli such as the presence of nutrients in the lumen (16, 19) and distension of the gut wall to adapt motility according to conditions within the lumen (for review see Ref. 5). Studies of the guinea pig ileum have identified at least 15 classes of myenteric neurons that can be distinguished by their morphologies, neurochemical codes, and functions. These include one or more populations of intrinsic sensory (or primary afferent) neurons, orally directed (ascending) and anally directed (descending) interneurons, and excitatory and inhibitory motor neurons supplying the circular and longitudinal muscle layers (9). However, the diversity of the ENS is such that the functions of some classes cannot always be clearly defined. For example, there are recent reports that some interneurons in the myenteric plexus of the guinea pig distal colon respond directly to stretch (39, 40). Furthermore, under some conditions the intrinsic sensory neurons of the small intestine may act as interneurons that coordinate the movement of complex motor patterns along the intestine in the absence of ongoing sensory input (43). However, despite this diversity, we now know much about the properties of individual enteric neurons but less about the nature of synaptic transmission at specific synapses within neural circuits that are activated by functional stimuli or stimuli from a known origin.

Previous studies have indicated that slow excitatory postsynaptic potentials (EPSPs) seen in myenteric neurons of the guinea-pig small intestine arise from diverse sources and are mediated by a variety of neurotransmitters. Different slow EPSPs are evoked by using single or multiple stimuli, and they can be described according to their time courses (seconds to minutes), pharmacology, and underlying conductance changes (for review see Ref. 17). Slow EPSPs mediated by 5-HT₇ receptors can be evoked in AH/Dogiel type II neurons by trains of electrical stimuli delivered to longitudinally running pathways (29). However, similar trains of stimuli delivered to circumferentially directed pathways evoke slow EPSPs mediated by tachykinins acting at neurokinin-1 (NK1) and NK3 receptors (2, 21). It is unclear whether the NK1- and NK3-mediated slow EPSPs arise from distinct pathways that can be differentially activated. Less is known about transmitters mediating slow EPSPs in myenteric S/Dogiel type I neurons. The most direct evidence indicates that slow EPSPs in inhibitory motor neurons are mediated by NK1 receptors (1, 44), whereas those in nitric oxide synthase (NOS)-immunoreactive (IR) descending interneurons are not mediated by NK1, NK3, or class 1 metabotropic glutamate receptors (44). Studies of reflexes have implicated acetylcholine acting at muscarinic receptors and a tachykinin acting at NK3 receptors at various sites in ascending reflex pathways (22, 45) and tachykinins acting at NK1 receptors in inhibitory motor neurons in descending reflex pathways (22). Studies of submucosal S neurons have identified two types of EPSPs mediated by P2Y₁ receptors that can be distinguished by their time courses. These are each evoked by a single electrical stimulus applied to an internodal strand and are termed slow EPSPs, which last 10–15 s, and intermediate EPSPs, which last 250–1,500 ms (20, 28). However, it is unknown whether P2Y₁ receptors also mediate EPSPs in myenteric neurons. These diverse observations highlight the need to examine which transmitters and receptors mediate different types of metabotropic transmission within well-defined neural pathways.

In this study, we used electrophysiological and immunohistochemical techniques to investigate neurotransmitters and receptors mediating slow EPSPs in myenteric AH and S neurons produced by activation of neural pathways originating in the mucosa.
MATERIALS AND METHODS

Guinea-pigs (180–380 g) of either sex were killed by stunning and having their carotid arteries and spinal cord severed. This procedure was approved by the University of Melbourne Animal Experimentation Ethics Committee. The abdominal cavity was opened, and segments of jejunum or ileum (5–10 cm in length) were removed, flushed clean, and placed in oxygenated (95% O2, 5% CO2) physiological saline (composition in mM: NaCl 118, KCl 4.6, CaCl2 2.5, MgSO4 1.2, NaH2PO4 1, NaHCO3 25, D-glucose 11) containing nicardipine (1.25 μM) and hyoscine (1 μM) to minimize contractions of any remaining circular muscle during intracellular recordings. The segment was opened along the mesenteric border and pinned flat in a dissecting dish lined with a silicone elastomer (Sylgard 184; Dow Corning, North Ryde, NSW, Australia). The mucosa, submucosa, and circular muscle were removed from one half of the preparation to reveal the myenteric plexus circumferentially adjacent to intact mucosa (26). The preparation was pinned into a recording bath (volume 1–2 ml), which was superfused with warmed physiological saline (35°C) at 5 ml/min and left to equilibrate for 1 h. Myenteric neurons were impaled using conventional intracellular recording techniques (4, 7). Impaled neurons were filled with 2% biocytin (Sigma Aldrich, Castle Hill, NSW, Australia) during recordings to allow morphological identification after the experiment. A unipolar stimulating electrode (65 μm stainless steel insulated with 15 μm Teflon) was used to deliver single pulses (1–2 mA, duration 0.5 ms) or trains of pulses (5 or 10 pulses, 20 Hz) to the mucosa circumferentially opposite the impaled ganglion (Master-8 stimulator, ISO-flex stimulus isolation unit; AMPI, Jerusalem, Israel). The responses to electrical stimulation were recorded at resting membrane potential (RMP) in AH neurons and over a range of membrane potentials in S neurons. In each type of neuron, mean EPSP amplitudes were obtained by measuring three to four control responses and calculating the average. The mean durations of EPSPs in S neurons were obtained by measuring the time from the start of the stimulus until the membrane potential returned to baseline. The durations of slow EPSPs in AH neurons were often quite variable, and it was difficult to determine when the membrane potential had returned to baseline; therefore these were not measured systematically. The pharmacology of slow EPSPs evoked by the different modes of electrical stimulation was examined by adding antagonists to the superfusing solution for 10–25 min. Each antagonist was washed from the bath solution for 20–30 min before a second antagonist was tested. Where an effect was seen with the first antagonist, one to two responses were recorded after this washout period to check for reversibility before the second antagonist was added. After each experiment, the morphology of impaled neurons and, where possible, immunoreactivity for NOS was examined using standard processing methods (18, 32).

Drugs. Drugs used were hyoscine, hexamethonium, pyridoxal phosphate-6-axophenyl-2-4-disulfonic acid (PPADS), nicardipine (all from Sigma Aldrich), MRS 2179 (Tocris Cookson, Bristol, UK), and SR142801 and SR140333 (gifts from Dr. Emonds-Alt, Sanofi Recherche, Montpellier, France). All drugs were dissolved in distilled water to make stock solutions and diluted to working concentrations in saline on the day of the experiment.

Analysis and statistics. Data are displayed as means ± SE unless otherwise stated. Statistical comparisons were made using paired t-tests. Values of P < 0.05 were considered statistically significant.

RESULTS

Trains of stimuli to the mucosa evoked slow EPSPs in AH neurons mediated by NK1 receptors. A train of electrical pulses (10 pulses, 20 Hz) delivered to the mucosa evoked a long duration (1–2 min) slow EPSP in 51 of 66 AH neurons impaled in 43 preparations (Fig. 1). The pharmacological properties of these slow EPSPs were studied in 11 of these neurons (mean RMP −54.5 ± 1.5 mV; range −62 to −49 mV). In 6 of the 11 neurons, SR142801 (100 nM) was added first followed by a 25-min wash period before SR140333 (100 nM) was added. SR142801 has been reported to have irreversible effects on NK3 receptors (10, 21, 34) and was only added once to each preparation. In another three neurons, SR140333 was added and washed out before adding SR142801. SR140333 only was investigated in the remaining two neurons. SR140333 abolished the slow EPSP in four neurons (Fig. 1D), significantly reduced it in six neurons (control 14.1 ± 2.2 mV, SR140333 3.6 ± 0.7 mV, P < 0.003, N = 6), and had no effect in one neuron. In contrast, SR142801 had little or no effect on the slow EPSPs in seven of the nine neurons for...
which it was tested (Fig. 1B), including the one insensitive to SR140333. However, in two cases where SR140333 had been added first and produced reversible reductions of 80% and 63% in slow EPSP amplitude, SR142801 subsequently produced a 25–30% reduction in slow EPSP amplitude. SR140333 appeared to be equally effective whether it was added first in the experiment or was added after SR142801. Reversibility of SR140333 was achieved after washout in three out of four neurons where impalements were maintained for long enough, including two occasions where it was added before SR142801. In the third experiment where SR140333 was added first, it had no effect. PPADS (30 μM, N = 5) and MRS 2179 (10 μM, N = 2) had no effect on these slow EPSPs. Intracellular injection of biocytin and subsequent processing to reveal the morphology of these neurons showed that they all had large cell bodies and several long processes, the characteristic morphology of Dogiel type II neurons.

Single pulse mucosal stimuli evoked slow depolarizations with different time courses in S neurons. A total of 66 S neurons from 40 preparations were studied (mean RMP −43.9 ± 1.1 mV; range −58 to −35 mV). Single stimuli applied to the mucosa evoked a fast EPSP, which triggered an action potential, followed by a slower depolarization in 35 of these neurons. These slower depolarizations ranged in amplitude from 3–12 mV when the RMP was held at −60 mV with hyperpolarizing current. Interestingly, their durations were shorter than the 10–30-s duration slow EPSPs that have been previously described in myenteric S neurons (1, 24, 33, 46). As can be seen in Fig. 2, most (26) of these slow depolarizations had durations of 300–900 ms, which were similar to the durations of the intermediate EPSPs seen in submucosal neurons, and we have called these responses intermediate EPSPs in DISCUSSION. The depolarizations in another nine neurons had durations of 1.3–9.0 s, which approaches those of the slow EPSPs reported previously, and they have been called slow EPSPs below.

Slow EPSPs were mediated by P2Y1 receptors and were predominantly in NOS-IR descending neurons. The slow EPSPs had a mean amplitude at −60 mV of 8.0 ± 0.9 mV and duration of 3.9 ± 1.1s, (N = 9, Fig. 3A). However, their amplitudes were not membrane potential dependent when tested over a range of resting potentials (−80 to −50 mV; N = 8, Fig. 4) induced by passing hyperpolarizing or depolarizing current through the recording electrode. No obvious changes in input resistance were observed during the slow EPSPs although

![Fig. 3](http://ajpgi.physiology.org/)

Fig. 3. Top, I: confocal image showing a myenteric S neuron injected with biocytin during electrophysiological recording and later stained with streptavidin Texas red. Top, II: same field showing both biocytin and nitric oxide synthase (NOS) immunoreactivity (green). The neuron was NOS positive. A: fast EPSP (grey arrow) followed by a slow EPSP (black arrow) evoked in the NOS immunoreactive neuron after a single-pulse stimulus to the mucosa. The slow EPSP was abolished by pyridoxal phosphate-6-axophenyl-2-4-disulfonic acid (PPADS) (30 μM, B) which was reversible on washout (Wash) (C). The fast EPSP was unaffected. D: slow EPSP was also abolished by MRS 2179 (10 μM).
or ramifying in the muscle, respectively. Motor neuron on the basis of side branches in more oral ganglia neurons negative for NOS immunoreactivity were identified as branches in ganglia or the muscle before it faded. The two motor neuron, whereas the other ran analy without apparent projection but could not be traced to its terminals and the projection of another neuron could not be determined before it faded.

In 31 of the 66 S neurons tested, neither intermediate nor slow EPSPs were evoked by individual stimuli although a fast EPSP was recorded. NOS immunoreactivity was examined in 21 of these neurons, and six were NOS positive. Two of the NOS-positive neurons were identified as inhibitory motor neurons, two were interneurons, and two had an anal projection but could not be traced to their terminals. The NOS-negative neurons included eight motor neurons and five interneurons, three with orally projecting axons, one projecting analy, and one circumferentially. Two of the NOS-negative neurons had anal projections but could not be traced to their terminals.

Responses of S neurons to short trains of mucosal stimuli. Trains of mucosal stimuli (5 pulses, 20 Hz) were tested in 44 of the 66 S neurons. Slow EPSPs (mean amplitude 19.4 ± 2.8 mV and duration 2.3 ± 0.5 s) were evoked in 10 of 16 neurons that also displayed intermediate EPSPs in response to single-pulse stimuli. Seven of these neurons were NOS negative, and two were NOS-positive descending interneurons (one neuron was not tested for NOS immunoreactivity). Five of the nine neurons that displayed single-pulse slow EPSPs were tested with a five-pulse stimulus, and three responded with a slow EPSP (mean amplitude 9.6 ± 0.9 mV). These had significantly

abolished by hexamethonium in six of six neurons. Fast EPSPs were sometimes seen superimposed on the intermediate EPSPs, but intermediate EPSPs themselves did not usually trigger action potentials. Morphological analysis of 21 of the 26 neurons revealed that they were a mixture of interneurons and motor neurons that were mostly NOS negative (N = 18, Fig. 6) but included three NOS-positive descending interneurons. Of the NOS-negative neurons, seven projected analy, four orally, and five had axons projecting circumferentially. One neuron had an oral projection but could not be traced to its terminals and the projection of another neuron could not be determined before it faded.

Intermediate EPSPs were mediated by P2Y1 receptors in neurons that were predominantly NOS negative. Intermediate EPSPs were evoked by single electrical stimuli to the mucosa in 26 of 66 S neurons and were defined as those with durations less than 1 s (mean amplitude at −60 mV; 6.9 ± 0.6 mV, duration 710 ± 30 ms, Fig. 6). PPADS (30 μM) reversibly blocked intermediate EPSPs in each of eight neurons tested (Fig. 6, A and B). This was confirmed to be via an action on P2Y1 receptors because MRS 2179 (10 μM) reversibly blocked the intermediate EPSP in another eight neurons (Fig. 6, C and D). The intermediate EPSPs were also preceded by a fast EPSP/action potential complex, and, when the membrane potential was hyperpolarized to −80 mV to prevent action potential firing, it was found that the fast EPSP was resistant to PPADS (control 28.8 ± 1.1 mV, PPADS 28.6 ± 1.1 mV, N = 8). However, the complex was
longer durations than the multipulse slow EPSPs seen in the neurons displaying intermediate EPSPs described above (mean duration 9.4 ± 2.7 s, p < .001). All three of these neurons were NOS positive, two were anally projecting interneurons, and one had an anal projection that could not be traced to its terminals.

Of the neurons that did not display a single-pulse EPSP (N = 31), 21 were tested with five-pulse stimulation, and six responded with slow EPSPs (mean amplitude 18.0 ± 4.6, duration 13.1 ± 7.8 s). NOS immunoreactivity was examined in three of these six neurons, and all three were NOS positive. None of the NOS-negative neurons tested (N = 11) responded to five-pulse stimulation, but ten-pulse stimulation evoked a slow EPSP in two of them (mean amplitude 8.5 ± 0.2 mV, mean duration 25.6 ± 2.7 s). The pharmacological properties of S neuron responses to trains of mucosal stimuli were not investigated.

Controlling for stimulus spread. To ensure that the stimuli used were not activating axons in either the myenteric plexus or the fiber bundles that run between the submucosa and the myenteric plexus, a series of control experiments were performed. The mucosa and submucosal layers were lifted to-
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shown that trains of mucosal stimuli excite pathways preferentially leading to NK1-mediated slow EPSPs in myenteric AH/Dogiel II neurons. Secondly, we have shown that single mucosal stimuli evoke P2Y1-mediated EPSPs that differ in time course between different types of S neurons. The shortest duration EPSPs are similar to the intermediate EPSPs seen in submucosal neurons and are seen predominantly in NOS-negative neurons. In contrast, the longer duration EPSPs are seen predominantly in NOS-positive anally projecting neurons.

Source of the synaptic potentials arising from mucosal stimuli. A key issue for the interpretation of the data presented here is where the stimuli are actually acting. It is possible that the stimuli are exciting axons in the mucosa itself, axons in the submucosal plexus, or axons in the myenteric plexus. It seems likely that most of the axons excited are in the mucosa although some stimulus spread to the submucosal plexus cannot be ruled out on the basis of the present results. Stimulus spread to the myenteric plexus or even to the axons that penetrate the circular muscle to connect the submucosa to the myenteric plexus (31, 38) is highly improbable. Maximal responses to mucosal stimuli were evoked by current pulses with amplitudes less than half those required to evoke minimal responses when connections between the layers were intact. However, this control experiment cannot rule out stimulus spread to the submucosal plexus. Nevertheless, spread to the submucosal plexus is unlikely to have such a consistent effect in producing synaptic responses in myenteric neurons, because Monro et al. (30) have shown that input from the submucosa to myenteric neurons is relatively sparse. Furthermore, the properties of the intermediate and slow EPSPs identified in S neurons after mucosal stimulation in the present study were distinctly different from the rare synaptic responses seen in myenteric neurons after stimulation of the submucosal plexus.

Trains of mucosal stimuli evoke NK1-mediated slow EPSPs in myenteric AH/Dogiel II neurons. Previous studies have shown that slow EPSPs evoked in myenteric AH/Dogiel II neurons by trains of electrical stimuli applied to circumferentially directed myenteric fiber tracts are mediated by tachykinins acting at either NK1 or NK3 receptors (2, 21). These slow EPSPs have been thought to arise predominantly by activation of the circumferentially projecting network of connections known to exist between myenteric AH neurons (27). However, our results show that activation of pathways probably originating in the mucosa preferentially evokes slow EPSPs mediated by NK1 tachykinin receptors in these neurons. This raises the possibility that NK1 receptor-mediated slow EPSPs in myenteric AH/Dogiel II neurons come from pathways anatomically distinct from those leading to NK3-mediated slow EPSPs. This is an exciting idea because a functional role for NK1-mediated slow EPSPs in these neurons has not previously been identified. One way this might occur is if inputs to myenteric AH neurons from submucosal AH neurons operate via NK1 receptors, while those inputs from other myenteric AH neurons operate via NK3 receptors. Both submucosal and myenteric AH neurons have projections to the mucosa and to each nerve plexus (12, 13), and these connections may be important for the coordination of motility and secretion. Thus the pathway activated by electrical stimulation leading to the NK1-mediated slow EPSPs in our study could involve the activation of either or both of these classes of neurons. However, recent electrophysiological evidence suggests that slow EPSPs evoked in myenteric AH/Dogiel II neurons by stimulation of a submucosal ganglion might be relatively rare (13%) (30), whereas in our study 77% of myenteric AH neurons responded with a slow EPSP from mucosal stimulation. There are two main possibilities that might account for this discrepancy. First, the stimuli may have antidromically activated myenteric neurons that subsequently triggered NK1-mediated slow EPSPs in the impaled myenteric AH neurons. Second, the stimuli used in the study by Monro et al. (30) may have been too localized to excite more than a small fraction of the axons projecting from cell bodies in the submucosal plexus to the myenteric plexus. Song et al. (37) estimated that each mucosal villus is innervated by at least 70 submucous neurons, 20% of which are IR for substance P, which labels submucosal AH/Dogiel type II neurons (6, 25). Thus the mucosal stimulation in our study might be expected to excite many more submucosal AH neurons than the focal stimulation applied in the study by Monro et al. The present data do not allow these two possibilities to be distinguished, but it is clear that the pathway coming from the mucosa is pharmacologically distinct from that excited by stimulation within the myenteric plexus. Furthermore, recent evidence has shown that activation of a third, longitudinally oriented, pathway leads to slow EPSPs in myenteric AH neurons mediated by 5-HT acting at 5-HT7 receptors (29), consistent with earlier suggestions using less specific methods (15, 46). Thus there appear to be at least three anatomically distinct pathways triggering slow EPSPs that converge on myenteric AH/Dogiel II neurons, and each acts via a different receptor subtype.

Computer simulation studies have suggested a functional role for slow metabotropic transmission within the circumferentially projecting network of connections between myenteric AH neurons. Presumably, this transmission is predominantly mediated by NK3 receptors, and it appears to be important in encoding the magnitudes of ongoing physiological stimuli (41, 42). These studies suggest that the after hyperpolarizing potentials (AHPs) characteristic of these neurons provide a source of negative feedback within the recurrent network. The AHPs are suppressed by slow EPSPs (46). The extent of this suppression can in turn set the gain of the circuit in relation to an ongoing stimulus. This may be one way myenteric AH neurons encode stimuli from different sources to initiate appropriate
functional responses. Identifying functional roles for the NK1-mediated slow EPSPs activated by mucosal pathways and 5-HT-mediated slow EPSPs in descending pathways should lead to a better understanding of how myenteric AH neurons coordinate intestinal motor patterns in response to different physiological stimuli.

Mucosal stimulation evokes P2Y1-mediated EPSPs that differ in time course between different types of S neurons. Mucosal stimulation evoked a depolarization in about half the S neurons studied, but the properties of this depolarization differed in many ways from slow synaptic responses previously reported for myenteric S neurons. The depolarizations were almost certainly synaptic potentials because they were abolished by either the relatively nonspecific P2 receptor antagonist PPADS and by the specific P2Y1 receptor antagonist MRS 2719, and therefore, they also appear to be mediated by metabotropic P2Y1 receptors. This is consistent with the very recent findings of Gallego et al. (14) that myenteric neurons exhibit P2Y1-mediated calcium transients in response to locally applied purines and electrical field stimulation. The durations of the EPSPs are markedly shorter than those of the nonmuscarinic slow EPSPs evoked by single-pulse stimulation of myenteric nerve trunks (mean duration 15 s) (24), which are thought to be mediated by tachykinins (23). Furthermore, the amplitudes of the EPSPs were not membrane potential dependent when tested over a range of resting potentials (Fig. 4), and no consistent changes in input resistance were observed during either type of EPSP. These observations suggest that a mixed conductance change might underlie the EPSP in each case although this was not tested directly in our study.

This idea is supported by a previous study showing that slow EPSPs can be attributable to simultaneous activation of K+ channels and Cl− channels in myenteric neurons (3). However, other reports in myenteric neurons describe an increase in slow EPSP amplitude at depolarized membrane potentials associated with an increase in membrane input resistance and reversal potentials equal to the equilibrium potential for potassium (24, 33). Furthermore, a study of slow EPSPs evoked by different neurotransmitters in submucosal neurons showed that they were all mediated by the activation of a sodium-selective cation current with inhibition of potassium conductances (36). Thus it is clear that the cellular mechanisms underlying slow EPSPs in enteric neurons vary depending on the neurotransmitter involved and the type of postsynaptic neuron.

Figure 2 clearly shows that the responses could be subdivided on the basis of their durations, with one group having durations from 300–900 ms and the other having durations of 1.3–9.0 s. By itself, this division might seem somewhat arbitrary, but the two types of response were also preferentially seen in different types of myenteric neurons. This contrasts with the submucosal plexus, where the intermediate EPSPs mediated by P2Y1 receptors are found in the same neurons that have P2Y1 receptor-mediated slow EPSPs (11, 28). Most neurons that exhibited the longer EPSPs were NOS-IR and projected anally with several being identifiable as interneurons. This supports the previous finding that slow EPSPs have been recorded in NOS-IR descending interneurons in response to distension orally (44). The durations of these slow EPSPs are closer to those of the muscarinic slow EPSPs (3–15 s) evoked by single-pulse stimuli reported previously by North and Tokimasa (33); however, our experiments were performed in the presence of a muscarinic antagonist. In contrast, the great majority of the neurons with the intermediate duration EPSPs were not NOS-IR although they could be identified from their projections as motor neurons and interneurons. These had similar durations (300–900 ms) to the intermediate EPSPs seen in submucosal neurons, which are also mediated by P2Y1 receptors (28). This is a novel finding because intermediate EPSPs have not previously been described in the myenteric plexus. Thus our results indicate that individual mucosal stimuli can evoke intermediate EPSPs and slow EPSPs in separate populations of myenteric S neurons.

Whereas mucosal stimulation evoked slow EPSPs predominantly in anally projecting NOS neurons, the intermediate EPSPs were evoked in a mixture of interneurons and motor neurons projecting orally, anally, and circumferentially. The intermediate EPSPs therefore appear to be more widespread in the myenteric plexus and are probably involved in both ascending and descending pathways activated by mucosal stimuli. Because intermediate EPSPs have previously not been described in the myenteric plexus and only relatively recently in the submucosal plexus, it is unclear what functional role they might play in reflex pathways. However, they are not likely to be involved in local reflex pathways activated by nutrient amino acids because MRS 2179 had no effect on inhibitory junction potentials (IJPs) evoked in the circular muscle by application of amino acids to the mucosa (16), and the latencies of these IJPs are generally shorter (150 < 300 ms) than the shortest duration intermediate EPSP we recorded in the present study. Their time courses lie in between those of fast EPSPs and slow EPSPs lasting tens of seconds; thus it is possible they could interact with fast synaptic transmission to cause a short lasting amplification of the neuronal response during high-frequency trains of fast EPSPs. Further experiments will be required to identify what role, if any, the myenteric and submucosal intermediate EPSPs play in synaptic transmission within reflex pathways activated by functional stimuli.

Another interesting question raised by this study is the reason for the difference in time course between the two different P2Y1 receptor-mediated synaptic potentials. In the submucosal plexus, this difference could be inferred to be related to the different conductance changes that underlie intermediate and slow EPSPs (11, 28); however, no obvious differences in underlying conductance were seen in myenteric neurons. Another possibility is that the differences between neuronal subtypes extend to either the handling of the intracellular messengers activated by P2Y1 receptors, usually thought to be coupled to phospholipase C (35), or the kinetics of the target ion channels, which have not yet been determined. Identification of these differences should prove an interesting challenge for future studies.

Conclusions. This study has shown that trains of mucosal stimuli excite pathways preferentially leading to NK1-mediated slow EPSPs in myenteric AH/Dogiel II neurons. Also, we have shown that single mucosal stimuli evoke P2Y1-mediated EPSPs that differ in time course between different types of myenteric S neurons. Future studies should focus on identifying functional roles for the different types of metabotropic transmission identified in pathways activated in the mucosa.
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GRANTS

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