ATP Mediates Fast Synaptic Potentials in Enteric Neurons

James J. Galligan and Paul P. Bertrand

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan 48824

Conventional intracellular electrophysiological methods were used to study fast synaptic transmission in the myenteric plexus of guinea pig ileum *in vitro*. Fast excitatory postsynaptic potentials (fEPSPs) were evoked in 98 neurons following single stimuli applied to interganglionic connectives. The nicotinic antagonist hexamethonium (100 μ M) reduced fEPSPs by 83% in 37 neurons; these fEPSPs were considered to be cholinergic. In 61 neurons, hexamethonium reduced fEPSPs by 33%; fEPSPs recorded in the presence of hexamethonium were considered to be noncholinergic. Similar data were obtained using the nicotinic antagonist mecamlamine (10 μ M) to block fEPSPs. Hexamethonium or mecamlamine completely blocked depolarizations caused by acetylcholine (ACh) applied by iontophoresis. The P_2 receptor antagonist suramin (1–300 μ M) inhibited noncholinergic fEPSPs in 30 cells; the suramin IC_{50} was 4 μ M. Suramin (100 μ M) did not block depolarizations caused by ACh or 5-HT, but suramin blocked depolarizations caused by ATP. Hexamethonium did not block ATP-induced depolarizations. The estimated reversal potential for suramin-sensitive fEPSPs and ATP-induced depolarizations was –25 and –16 mV, respectively. ATP responses were reduced in low-sodium (26 mM) extracellular solution, suggesting that ATP activates a cation channel. These data indicate that in myenteric nerves ATP, in addition to ACh, contributes to fast synaptic transmission.

[Key words: enteric nerves, synaptic transmission, purine receptors, ligand-gated ion channels, autonomic nervous system, gastrointestinal tract]

ATP is a cotransmitter released with norepinephrine from sympathetic nerves that mediate contractions of some smooth muscle (Burnstock and Kennedy, 1986; von Kugelgen and Starke, 1991). ATP is an agonist at P_2 purine receptors (Burnstock and Kennedy, 1985) and in smooth muscle cells, ATP acts on P_2 receptors to cause depolarization and contraction (Benham et al., 1987; Friel, 1988). Some P_2 receptors are likely to be ligand-gated cation channels, as they are permeable to sodium, potassium, and calcium and mediate short-latency, short-duration responses (Benham et al., 1987; Friel, 1988). ATP also acts at P_2 receptors on sensory neurons (Krishtal et al., 1988; Bean, 1990), autonomic neurons (Allen and Burnstock, 1990; Fieber and Adams, 1991b; Nakazawa, 1994), and neurons in the CNS

(Jahr and Jessell, 1983; Ueno et al., 1992; Shen and North, 1993) to cause responses similar to those caused by acetylcholine (ACh) acting at nicotinic receptors. ATP-activated ion channels are present in cell-free membrane patches from neurons, indicating a close coupling between the ATP binding site and the cation channel (Krishtal et al., 1988; Bean et al., 1990; Silinsky and Gerzanich, 1993). Fast excitatory postsynaptic potentials (fEPSPs), mediated by ATP acting at P_2 receptors, have been recorded from guinea pig celiac ganglion neurons (Evans et al., 1992; Silinsky et al., 1992) and from neurons in the medial habenula of the rat (Edwards et al., 1992).

The enteric nervous system (ENS) is the division of the autonomic nervous system residing in the wall of the gastrointestinal tract (Langley, 1921). The ENS is composed of many neurochemically and functionally different neurons that contain a variety of potential neurotransmitters and express receptors for these substances (Furness and Costa, 1987; Wood, 1989). ACh, acting at nicotinic receptors, is the principal excitatory neurotransmitter in the gut and ACh is believed to be the sole mediator of enteric fEPSPs (Wood, 1989). However, there are other substances contained in the ENS that cause responses similar to nicotinic responses. 5-Hydroxytryptamine (5-HT), acting at 5-HT₃ receptors, causes short-latency, short-duration depolarizations that are due to an increase in a cation conductance (Mawe et al., 1986; Surprenant and Crist, 1988) and 5-HT-activated channels are present in cell-free patches of enteric neurons (Derkach et al., 1989). In the ENS, ATP also causes fast inward currents and activates cation channels in cell-free patches of enteric nerves (Bertrand and Galligan, 1992; Barajas-Lopez et al., 1993, 1994). Although receptors mediating responses similar to nicotinic responses are present in the ENS, enteric noncholinergic fEPSPs have not been identified (Evans and Surprenant, 1992).

In the present study, conventional electrophysiological methods were used to record fEPSPs from myenteric neurons of guinea pig ileum *in vitro*. Acutely isolated preparations of myenteric plexus were used, as most synaptic connections present in the intact intestine are preserved. We have shown that ATP mimics fEPSPs and that the P_2 receptor antagonist suramin (Dunn and Blakely, 1988; Hoyle et al., 1990) blocks some fEPSPs. We conclude that ATP is a mediator of noncholinergic fEPSPs in the ENS.

Materials and Methods

Tissue preparation. Male guinea pigs weighing 250–350 gm were anesthetized via halothane inhalation, stunned, and bled through the neck. A segment of ileum was excised 10–20 cm proximal to the ileocecal junction and placed in oxygenated (95% O₂/5% CO₂) Krebs solution of the following composition (millimolar): NaCl, 117; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 25; NaH₂PO₄, 1.2; glucose, 11. The Krebs solution contained nifedipine (1 μ M) and scopolamine (1 μ M) to block

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Correspondence should be addressed to Dr. James J. Galligan, Department of Pharmacology and Toxicology, Life Sciences Building B440, Michigan State University, East Lansing, MI 48824.

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longitudinal muscle contractions and muscarinic receptors on myenteric nerves during intracellular recordings. A 1.5 cm segment of ileum was cut open along the mesenteric border and pinned out flat (mucosal surface up) in a petri dish lined with Silastic elastomer. A longitudinal muscle–myenteric plexus preparation was made by stripping away the mucosal, submucosal, and circular muscle layers using fine forceps and scissors. A 5 mm² piece of longitudinal muscle myenteric plexus was transferred to a small recording chamber (2 ml volume) lined with Silastic elastomer. The preparation was stretched lightly and pinned to the chamber bottom using small stainless steel pins. The preparation was superfused with 36°C, oxygenated Krebs solution at a flow rate of 7 ml/min.

Intracellular recording. Individual myenteric ganglia were visualized at 200× magnification using an inverted microscope (Nikon Diaphot or Olympus CK-2) with differential interference contrast optics (Hoffman Modulation). Intracellular recordings were obtained from single neurons using glass microelectrodes filled with 2 M KCl (tip resistance, 80–120 MΩ). An amplifier with an active bridge circuit (Axoclamp 2A, Axon Instruments, Foster City, CA) was used for membrane potential recordings and for injection of current into neurons via the recording microelectrode. In most experiments, the membrane potential was adjusted to a potential negative to −70 mV using constant DC current to avoid action potentials when evoking fEPSPs. Signals were filtered at 1 kHz using a 4-pole Bessel filter (Warner Instruments, New Haven, CT), digitized at 1 kHz using a Labmaster TL-1 or Digitdata 1200 analog/digital converter (both from Axon Instruments) and acquired and stored using AXOTAPE 2.0 (Axon Instruments) and a personal computer.

Synaptic potentials were evoked using a broken-back glass pipette (tip diameter 40–60 μm) filled with Krebs solution as a focal stimulating electrode. The stimulating electrode was positioned on an interganglionic nerve strand. To evoke an fEPSP, nerve fibers were stimulated using single stimuli of 0.3 msec duration applied at a rate of 0.2 Hz. When studying fEPSPs, 6–12 individual responses were averaged. In some experiments, slow excitatory postsynaptic potentials (sEPSPs) were evoked using trains of stimuli (20 Hz for 500 msec). The amplitude of single sEPSPs before and after drug treatment was measured.

Drug application. Antagonists were applied by superfusion in a known concentration by addition to the superfusing Krebs solution. Antagonists were applied for 4–12 min prior to measuring the amplitude of the evoked response. When constructing antagonist concentration–response curves, successive antagonist concentrations were applied cumulatively and each concentration was present for a minimum of 4 min before measuring the amplitude of the evoked response. ACh was applied by iontophoresis from an electrode (tip resistance < 20 MΩ) placed directly above the impaled neuron. The concentration of ACh in the electrode was 1 M. A retaining current of −6 nA was used and cathodal current pulses of 1–20 msec duration and 100–199 nA intensity were used. An average of 6–12 individual ACh responses was obtained for each treatment. ATP was applied by pressure ejection (10 psi) from a fine-tipped (<10 μm) pipette positioned within 200 μm of the impaled neuron. The concentration of ATP in the pipette was 100 or 300 μM.

Statistics. All data are expressed as the mean ± SEM. Student's *t* test for paired data or analysis of variance were used to establish significant differences between control and treatment groups. Antagonist inhibition of EPSPs, ACh, or ATP responses is expressed as percentage change from control amplitude. Concentration–response curves were plotted and concentrations of antagonist causing half-maximal inhibition (IC₅₀) were determined from average concentration–response curves.

Drugs. Mecamylamine was obtained from Research Biochemicals Inc. (Natick, MA). Suramin was a gift from the Center for Disease Control (Atlanta, GA). All other drugs and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Results

Nicotinic receptor antagonists, but not suramin, block ACh-induced depolarizations

ACh was applied by iontophoresis to cause a depolarization that was similar in amplitude and time course to fEPSPs recorded from myenteric neurons (Fig. 1*A*). Hexamethonium inhibited ACh-induced depolarizations in a concentration-dependent manner and the ACh response was completely blocked by 100 μM hexamethonium (Fig. 1*A,B*). The IC₅₀ for hexamethonium

was 3 μM at a membrane potential of -88 ± 5 mV ($n = 9$). Similar experiments were done using the nicotinic antagonist mecamylamine, which also inhibited ACh-induced depolarizations in a concentration-dependent manner (Fig. 1*B*). Mecamylamine (10 μM) completely blocked the ACh response and the mecamylamine IC₅₀ was 0.12 μM at a membrane potential of -88 ± 6 mV ($n = 7$). These data indicate that hexamethonium and mecamylamine can completely block nicotinic receptors activated by ACh when it is applied in a manner that mimics the amplitude and duration of fEPSPs.

ACh was applied by iontophoresis in the absence and presence of the P₂ receptor antagonist suramin (Fig. 2*A*). Suramin did not change the amplitude of the ACh response in any neuron tested (Fig. 2*B*). Furthermore, suramin (100 μM) did not block depolarizations caused by 5-HT (control, 23 ± 1 mV; suramin, 23 ± 5 mV; $n = 2$). The 5-HT depolarizations were blocked by the 5-HT₃ receptor antagonist ondansetron (1 μM) (control, 26 ± 4 mV; ondansetron, 6 ± 4 mV; $n = 3$, $p < 0.01$).

Noncholinergic fEPSPs in myenteric neurons

Single electrical stimuli applied to interganglionic nerve strands evoked an fEPSP in 98 neurons; the average amplitude of the fEPSP was 23 ± 4 mV. None of these neurons exhibited a spike afterhyperpolarization that lasted longer than 1 sec, and these cells would be classified as “S” neurons according to the criteria of Hirst et al. (1974). Hexamethonium (100 μM) reduced the fEPSP by $83 \pm 1\%$ in 37 neurons; in the presence of hexamethonium the fEPSP was 4 ± 0.4 mV at a membrane potential of -90 ± 4 mV (Fig. 3*A*, left). These fEPSPs were considered to be cholinergic. In 61 neurons, hexamethonium (100 μM) reduced the fEPSP by $34 \pm 3\%$; in these cells, fEPSP amplitude was 15 ± 1 mV in the presence of the antagonist at a membrane potential of -97 ± 3 mV. Hexamethonium-resistant fEPSPs were completely blocked by tetrodotoxin (0.3 μM, $n = 9$) (Fig. 3*A*, right). The control distribution of fEPSP amplitude was dispersed over a range of amplitudes between 10 and 40 mV (Fig. 3*B*). The distribution of fEPSP amplitudes in the presence of hexamethonium was skewed towards smaller amplitudes with a peak at less than 10 mV (Fig. 3*C*). There was a large overlap of the two distributions indicating that most fEPSPs in the myenteric plexus are a mixture of hexamethonium-sensitive and -insensitive components.

Although it was shown above that 100 μM hexamethonium completely blocked responses to ACh, it is possible that iontophoretically applied ACh and neurally released ACh act at different sites and that higher concentrations of hexamethonium may be required to block nicotinic fEPSPs. This differential sensitivity of receptors could account for the occurrence of hexamethonium-resistant fEPSPs. This possibility was tested in two ways. First, in 12 neurons in which the fEPSP was partly reduced by 100 μM hexamethonium, it was found that a higher concentration of hexamethonium (300 μM) did not further reduce the fEPSP (Fig. 4*A,B*). Second, mecamylamine was also used to determine if some fEPSPs recorded from myenteric neurons were noncholinergic. In 4 of 11 neurons, mecamylamine (10 μM) reduced the fEPSP by $77 \pm 2\%$ (Fig. 4*C*), while in 7 of 11 neurons, mecamylamine reduced the fEPSP by only $32 \pm 9\%$ (Fig. 4*D*). Taken together, these data indicate that the failure of 100 μM hexamethonium to block completely the fEPSP in some neurons cannot be attributed to the use of an insufficient concentration of antagonist or to a population of hexamethonium-resistant nicotinic receptors. Instead, the fEPSP in most myen-

Figure 1. Nicotinic receptor antagonists block ACh-induced depolarizations of myenteric neurons. *A*, ACh applied by ionophoresis (2 msec, 100 nA) to a myenteric neuron causes a depolarization that is inhibited by 10 and 100 μM hexamethonium. *B*, Concentration-response curves for inhibition of ACh-induced depolarizations by hexamethonium and mecamylamine. Data obtained from experiments similar to that shown in *A* and are the mean $\pm$ SEM of three to nine cells per point.

teric neurons is due to the action of a neurotransmitter or neurotransmitters in addition to ACh and the hexamethonium-resistant fEPSP is a noncholinergic synaptic event. The data presented in Table 1 show that the latency, time to peak, and time to half-decay for noncholinergic fEPSPs were similar to the time course of nicotinic fEPSPs.

Noncholinergic fEPSPs blocked by the P_2 receptor antagonist suramin

Suramin (100 μM) was applied to 35 of 61 neurons in which the fEPSP was partly reduced by hexamethonium (100 μM). In five

Figure 2. Suramin does not alter ACh-induced depolarizations. *A*, ACh applied by ionophoresis (5 msec, 199 nA) causes a depolarization that is not reduced in amplitude by suramin. *B*, Data from experiments similar to *A* demonstrating that suramin (300 μM) does not inhibit ACh responses in myenteric neurons ($n = 6$).

cells, suramin reduced the noncholinergic fEPSP by less than 20%. In 30 cells, suramin (100 μM) reduced the noncholinergic fEPSP by $71 \pm 4\%$ (Fig. 5*A*). The control noncholinergic fEPSP was 17 ± 1 mV, and in the presence of suramin the fEPSP was 5 ± 1 mV ($p < 0.01$). Inhibition of the noncholinergic fEPSP by suramin was concentration dependent; the suramin IC_{50} was 4 μM (Fig. 5*B*). Although it was shown above that suramin did not block responses to ACh or 5-HT, it is possible that suramin could act nonspecifically to disrupt synaptic transmission. This possibility is unlikely because there were some noncholinergic fEPSPs that were unaffected by suramin and it was also found that suramin (100 μM) did not change the amplitude of sEPSPs elicited by brief trains of stimuli (control sEPSP, 22 ± 4 mV; sEPSP in the presence of suramin, 17 ± 3 mV; $n = 5$, $p > 0.05$).

Table 1. Time course of cholinergic and noncholinergic fEPSPs in myenteric neurons

	Latency (msec)	Time to peak (msec)	Half-decay (msec)	Amplitude (mV)
Cholinergic fEPSP ($n = 11$)	3.1 ± 0.4	4.0 ± 0.6	13.1 ± 2.5	20.7 ± 2.5
Noncholinergic fEPSP ($n = 10$)	3.5 ± 0.2	4.1 ± 0.6	12.3 ± 1.2	20.6 ± 1.2

Data are mean $\pm$ SEM. There were no differences between cholinergic and noncholinergic fEPSPs ($p > 0.05$, Student's *t* test).

Figure 3. Cholinergic and noncholinergic fEPSPs. *A*, Left, fEPSP that was completely blocked by hexamethonium. Right, Recording from a different neuron with an fEPSP that was partly reduced in amplitude by hexamethonium. The noncholinergic fEPSP was completely blocked by tetrodotoxin (TTX). *B*, Amplitude-frequency distribution for fEPSPs recorded in the absence of hexamethonium. *C*, Amplitude-frequency distribution for fEPSPs recorded in the presence of hexamethonium (100 μ M).

The reversal potential for the noncholinergic fEPSP was determined in experiments similar to that shown in Figure 6, *A* and *B*. The amplitude of the suramin-sensitive fEPSP was measured at different membrane potentials in the presence of hexamethonium (100 μ M). In this cell, the estimated reversal potential was -15 mV (Fig. 6*C*). This experiment was repeated in six additional neurons and the average estimated reversal potential was -25 ± 7 mV. This value was similar to the estimated reversal potential for fEPSPs measured in the absence of hexamethonium (-24 ± 6 mV, $n = 8$).

ATP mimics noncholinergic fEPSPs

ATP was applied by pressure ejection from a micropipette onto neurons exhibiting noncholinergic fEPSPs. ATP caused a depolarization of 1–2 sec duration and the depolarization was

associated with a decrease in membrane input resistance (Fig. 7*A*). In 8 of 10 neurons in which the fEPSP was suramin sensitive, ATP caused a fast depolarization. ATP caused a fast depolarization in only one of eight neurons in which the fEPSP was completely blocked by hexamethonium. The fast depolarization caused by ATP was blocked by suramin (100 μ M) (Fig. 7*B*). Hexamethonium (100 μ M) did not block ATP-induced depolarizations (control, 22 ± 4 mV; hexamethonium, 26 ± 3 mV; $n = 2$). In some cells, ATP also caused a slow depolarization (Fig. 7*A*), but this response was not studied.

The reversal potential for the ATP response was estimated in experiments similar to that shown in Figure 8 where the amplitude of the ATP response was measured at different membrane potentials. In the experiment shown in Figure 8, the estimated reversal potential was -2 mV. Similar experiments

Figure 4. Effects of hexamethonium and mecamylamine on fEPSPs. *A*, fEPSP reduced in amplitude by 100 μM hexamethonium and 300 μM hexamethonium does not further reduce fEPSP amplitude. *B*, Data from experiments similar to that shown in *A* ($n = 12$). Hexamethonium at 100 and 300 μM significantly reduced the amplitude of the fEPSP ($p < 0.05$) but the effects of 100 μM are not different from the effects of 300 μM ($p > 0.05$). Data were analyzed by ANOVA and the least significant difference test.

were repeated in three additional neurons, and the average estimated reversal potential was -16 ± 5 mV. The decrease in input resistance during the ATP response and the reversal potential near 0 mV suggests that the ATP response in myenteric neurons may be due to an increase in a nonspecific cation conductance. Consistent with this suggestion, it was found that reducing the extracellular sodium concentration from 145 mM to 26 mM (choline chloride substitution for sodium chloride) shifted the reversal potential to -31 ± 1 mV ($n = 3$) (Fig. 8*B*).

Discussion

Noncholinergic fast synaptic transmission in the myenteric plexus

There are many potential neurotransmitters contained in the ENS (Furness and Costa, 1987). In addition to ACh, these potential neurotransmitters include 5-HT, GABA, nitric oxide, and many neuropeptides. Studies of the contribution of noncholinergic neurotransmitters to synaptic transmission in the ENS have focused on slow synaptic events and it has been assumed that ACh is the sole mediator of fast synaptic transmission between enteric nerves (Wood, 1989). However, in the present study, we have shown that in most myenteric neurons of guinea pig small intestine fEPSPs are mediated by neurotransmitters in addition to ACh acting at nicotinic receptors. This conclusion is based on the observation that most fEPSPs were not completely blocked by the nicotinic antagonist hexamethonium.

The failure of hexamethonium to block the fEPSP was not due to the use of insufficient concentrations of antagonist. ACh was applied by ionophoresis onto neurons, and the ACh response was similar in time course and amplitude to the fEPSP. This observation indicates that ionophoretically applied ACh and neurally released transmitter were acting at similar sites to cause a depolarization. The concentration of hexamethonium (100 μM) used to study fEPSPs was 30-fold higher than its IC_{50} value (3 μM) determined in studies of the ACh response, and at a concentration of 100 μM hexamethonium completely blocked ACh responses. In addition, in neurons in which 100 μM hexamethonium did not completely block the fEPSP, a higher concentration of hexamethonium (300 μM) did not further reduce the amplitude of the fEPSP.

The fEPSPs that were not blocked by hexamethonium were not due to an action of neurally released ACh at a population of hexamethonium-insensitive nicotinic receptors. Mecamylamine, another antagonist of ganglionic nicotinic receptors (Fieber and Adams, 1991a), was used to determine if some nicotinic receptors in the ENS were resistant to hexamethonium. Mecamylamine was an effective antagonist of ACh responses, and the concentration of mecamylamine (10 μM) used to study fEPSPs was almost 100-fold higher than the IC_{50} value (0.12 μM) determined for block of the ACh response. At a concentration 10 μM , mecamylamine did not block all fEPSPs recorded from myenteric neurons. In addition, mecamylamine and hexamethonium are open channel blockers of the nicotinic receptor,

Figure 5. Suramin inhibits noncholinergic fEPSPs. *A*, In the presence of hexamethonium ($100 \mu\text{M}$) suramin at 10 and $100 \mu\text{M}$ reversibly inhibits the noncholinergic fEPSP. Wash indicates recovery from suramin; hexamethonium was present throughout. *B*, Concentration–responses curve for inhibition of noncholinergic fEPSP by suramin. Data are the mean $\pm$ SEM of the indicated number of cells per data point.

and the block caused by these drugs is enhanced at hyperpolarized membrane potentials (Ascher et al., 1979; Fieber and Adams, 1991a). We studied the action of hexamethonium and mecamylamine on fEPSPs at potentials near -90 mV, which would increase the block of the nicotinic receptor produced by these drugs when compared to their effects at potentials near rest (-60 mV). Finally, there was a subpopulation of neurons in which hexamethonium or mecamylamine reduced the fEPSP by more than 80%, indicating that some fEPSPs were largely mediated by neurally released ACh acting at nicotinic receptors and that the nicotinic antagonists can block these receptors.

The noncholinergic response was completely blocked by tetrodotoxin, indicating that it was dependent on presynaptic action potentials. The latency, rise time, and decay time of the noncholinergic response were similar to the observed times for fEPSPs that were blocked by the nicotinic receptor antagonists. Therefore, the noncholinergic response is likely to be due to the action of a neurotransmitter at a ligand-gated ion channel similar to the nicotinic receptor. These data indicate that when nicotinic receptors are blocked by hexamethonium or meca-

mylamine, the remaining fEPSPs are noncholinergic. The marked overlap of the frequency distributions of fEPSP amplitudes in the absence and presence of hexamethonium indicates that most fEPSPs elicited by electrical stimulation in the ENS are due to the action of a transmitter or transmitters in addition to ACh.

ATP mediates noncholinergic fEPSPs

ATP acts at P_2 receptors on smooth muscle cells (Benham et al., 1987; Friel, 1988), sensory neurons (Krishtal et al., 1988; Bean, 1990), autonomic neurons (Fieber and Adams, 1991b; Nakazawa, 1994), and neurons in the CNS (Ueno et al., 1992; Shen and North, 1993) to cause a depolarization or inward current. The short latency, time course, and reversal potential (near 0 mV) of these responses have led to the conclusion that the purine receptor at which ATP acts is a ligand-gated cation channel with properties similar to the nicotinic receptor (Bean, 1992). The P_2 receptor antagonist suramin (Dunn and Blakely 1988; Hoyle et al., 1990) blocks ATP-induced responses in peripheral neurons (Nakazawa, 1994) and PC12 cells (Nakazawa et al., 1991a). Suramin has also been used to show that synaptic responses recorded from medial habenula neurons (Edwards et al., 1992) and neurons from guinea pig celiac ganglion in tissue culture are mediated by ATP (Evans et al., 1992; Silinsky and Gerzanich, 1993). In the present study, suramin reduced noncholinergic fEPSPs in 30 of 35 neurons tested. The inhibition of noncholinergic fEPSPs by suramin was concentration dependent and, on average, suramin reduced the noncholinergic component of the fEPSP by a maximum of 70%.

In neurons exhibiting a suramin-sensitive fEPSP, ATP caused a fast depolarization (<2 sec duration), and the fast depolarization was associated with a decrease in input resistance. ATP generally did not cause a fast depolarization in neurons in which the fEPSP was blocked by hexamethonium. The depolarization caused by ATP was blocked by suramin but suramin did not block ACh responses or depolarizations caused by 5-HT acting at 5-HT_3 receptors. Depolarizations caused by 5-HT were blocked by the 5-HT_3 receptor antagonist ondansetron (Derkach et al., 1989; Vanner and Surprenant, 1990). Suramin did not block noncholinergic fEPSPs via a nonspecific action on synaptic transmission, as some noncholinergic fEPSPs were not blocked by suramin and slow synaptic potentials evoked by trains of stimuli were not affected by suramin.

In other cells, ATP activates a nonspecific cation channel as ATP-induced currents reverse polarity near 0 mV and reducing extracellular sodium shifts the reversal potential in a negative direction (Benham et al., 1987; Friel, 1988; Krishtal et al., 1988; Fieber and Adams, 1991b; Nakazawa et al., 1991b). As the ATP response recorded from myenteric neurons was associated with a decreased input resistance, the ATP response and noncholinergic fEPSP could be due to activation of a cation conductance. The estimated reversal potential for the noncholinergic fEPSP (-25 mV) and the ATP response (-16 mV) were similar and were not different from that measured for the nicotinic fEPSP (Wood, 1989). These data are consistent with the noncholinergic fEPSP and ATP responses being due to the activation of a nonspecific cation channel similar to the nicotinic receptor. However, our estimates of the ATP reversal potential and the reversal potential of the noncholinergic fEPSP are more negative than those obtained by others for ATP responses and suramin-sensitive synaptic responses (Benham et al., 1987; Friel, 1988; Bean et al., 1990; Fieber and Adams, 1991b; Edwards et al., 1992; Evans et al., 1992; Nakazawa, 1994; Silinsky and Ger-

Noncholinergic fEPSP

Figure 6. Reversal potential for suramin-sensitive fEPSP. *A*, Suramin reduces the amplitude of the noncholinergic fEPSP. *B*, Amplitude of the suramin-sensitive fEPSP measured at the indicated membrane potentials. *C*, Plot of data shown in *B*; the estimated reversal potential for the fEPSP in this cell was -15 mV.

ATP response

Figure 7. ATP mimics the noncholinergic fEPSP. *A*, Depolarization caused by ATP (triangle) applied by pressure ejection from a micropipette. Downward deflections are voltage responses to 200 pA, 100-msec-duration hyperpolarizing current pulses passed through the recording microelectrode. Decrease in amplitude of these voltage re-

sponses indicates a decrease in input resistance during the ATP response. *B*, ATP responses are blocked by suramin. Asterisk indicates significantly different from control ($p < 0.05$, $n = 6$).

zanich, 1993). This discrepancy may be due to the inward rectification exhibited by ATP-induced currents and suramin-sensitive fEPSPs. We used high-resistance intracellular microelectrodes (80–120 M Ω). Therefore, the amplitude of noncholinergic fEPSPs and ATP responses could be measured at potentials only as positive as -20 mV, and the reversal potential was estimated by extrapolation. As we did not measure the ATP response or the noncholinergic fEPSP in the range of potentials where ATP currents rectify, it is likely that our estimate of the reversal potential is more negative than the actual reversal potential. Alternatively, the properties of the ATP-activated channel in myenteric neurons may be different from the same channels in other cell types.

The ATP response was reduced when it was studied in low extracellular sodium solutions (26 mM). In low-sodium solutions, the ATP reversal potential shifted to -30 mV; this shift is less than that would be predicted when reducing the extracellular sodium concentration by 80%. Under these conditions, the ATP reversal potential should have shifted in the negative direction by 50 mV. The smaller shift measured here could be attributed to the rectifying properties of the ATP-activated channel or the channel in myenteric neurons may be somewhat permeable to choline and other cations including calcium, as shown in other cell types (Benham et al., 1987; Friel, 1988; Fieber and Adams, 1991b; Nakazawa et al., 1991b; Ueno et al., 1992).

In some neurons, the fast depolarization caused by ATP was followed by a slow depolarization. The slow depolarization caused by ATP in enteric neurons has been shown by others to be due to an inhibition of resting potassium channels (Katayama and Morita, 1989). It remains to be determined if there are slow synaptic potentials in the ENS that are also mediated by ATP.

Figure 8. Reversal potential for ATP response. *A*, Amplitude of ATP response at the indicated membrane potentials. *B*, Plot of data shown in *A*. In this cell, the estimated reversal potential was -2 mV. In the same cell, the reversal potential for the ATP response shifted to -30 mV when the sodium concentration was reduced to 26 mM (choline chloride substitution for sodium chloride).

Significance of noncholinergic fEPSPs

ATP induces a depolarization in many neurons *in situ* and in culture, but the functional significance of the ATP-induced response in these neurons is not clear (Bean, 1992). Recently, it has been shown that when glutamate and GABA receptors are blocked, fast synaptic currents recorded from medial habenula neurons are mediated by ATP (Edwards et al., 1992). As these studies were done in acutely isolated brain slices, the synaptic responses recorded in these studies are likely to be present *in vivo*. Celiac ganglion neurons maintained in tissue culture for 1 week or more form autapses which use ATP as the excitatory neurotransmitter (Evans et al., 1992; Silinsky and Gerzanich, 1993). Although occasional ATP-mediated synaptic responses occur in the intact celiac ganglion (Silinsky and Gerzanich, 1993), most fEPSPs in this tissue are blocked by nicotinic antagonists, and the functional significance of ATP-mediated synaptic potentials recorded in tissue culture are unknown (McLachlan and Meckler, 1989; McLachlan and Keast, 1993). We used acutely isolated preparations of myenteric plexus, and most synaptic connections present in the intact intestine would be preserved in this preparation. Therefore, the ATP-mediated fEPSPs recorded here are likely to contribute to ganglionic neurotransmission in intact tissues. Histochemical methods using quina- crine to reveal neuronal stores of ATP have demonstrated that quinacrine-induced fluorescent nerve fibers and nerve cell bodies are found throughout the myenteric plexus of the small intestine of mouse, guinea pig, and rat (Olson et al., 1976). In addition, synaptosomes prepared from the myenteric plexus of

guinea pig small intestine release ATP in a calcium-dependent manner (White and Leslie, 1982). These histochemical and neurochemical data are consistent with the electrophysiological data presented here indicating that ATP is a fast synaptic transmitter in the ENS.

Reflex peristaltic contractions can be elicited *in vitro* in isolated segments of intestine when the lumen of the segment is slowly filled with fluid (Trendelenburg, 1917). The peristaltic reflex involves a filling phase in which the intestinal segment accommodates to the volume of fluid and an emptying phase that occurs when intraluminal pressure has reached a critical level. The emptying phase of the peristaltic reflex is generally blocked by nicotinic antagonists (Furness and Costa, 1987). However, distention-evoked peristalsis in guinea pig ileum can be elicited in the presence of hexamethonium when the tissues are also incubated with the opioid receptor antagonist naloxone (Bartho et al., 1987). Recently, it has been shown that at low rates of distention, the nerve-mediated accommodation that occurs during the filling phase of the peristaltic reflex is not blocked by hexamethonium (Waterman et al., 1994). Both groups concluded that non-nicotinic ganglionic transmission was responsible for the hexamethonium-resistant reflexes they had described. Although previous work dealing with noncholinergic ganglionic transmission in the ENS has focused on slow synaptic transmission (Wood, 1989), it is possible that ATP acting as a fast synaptic transmitter mediates these hexamethonium-resistant reflexes.

In conclusion, ATP-mediated fEPSPs contribute to excitatory ganglionic transmission in the myenteric plexus of guinea pig

small intestine. It remains to be determined which of the many functionally distinct myenteric nerves release ATP as a neurotransmitter and which neurons receive this excitatory purinergic input. Finally, we have identified noncholinergic fEPSPs that were not blocked by suramin. There are likely to be mediators of fast synaptic transmission in the ENS in addition to ACh and ATP.

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